UNITED STATES DEPARTMENT OF AGRICULTURE AGRICULTURAL RESEARCH SERVICE	Report To SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION NEW ORLEANS, LOUISIANA

CONTRACT PROJECT REPORT
(To be used as cover sheet for report)

Contracting Organization	Project Leader	
University of Florida	E. M. Ahmed	
Department	USDA Designated Representative	
Food Science	P. E. Shaw	
Section	Contract Number	Initiation Date
	12-14-100-10337	6/23/70
Locality	Report Number	Period Covered
Gainesville, Florida	12	6/23/70 - 3/23/73

TYPE OF REPORT: PROGRESS ( ) PHASE ( ) ANNUAL ( ) FINAL (XX)

Project Title:

Flavor enhancing potential of selected orange oil and essence components and their relationship to product quality.

ABSTRACT OF PROGRESS

NOT FOR PUBLICATION

2490

FLAVOR ENHANCING POTENTIAL OF SELECTED ORANGE OIL AND ESSENCE COMPONENTS AND THEIR RELATIONSHIP TO PRODUCT QUALITY

Prepared by
E. M. Ahmed
Department of Food Science
University of Florida
Gainesville, Florida

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

Final Report
For the Contract Period
June 23, 1970 to March 23, 1973

August, 1975

INTRODUCTION

Citrus is Florida's largest agricultural commodity. The on-tree sale value for the 1970-71 season was 285 million dollars. Oranges accounted for 71% of this citrus crop, or 142 million boxes. This represented about 24% of the world's orange production. Over 90% of the crop was processed and 73% (103 million boxes) were concentrated into 125 million gallons of FCOJ.

During the preparation of FCOJ, the juice is mechanically squeezed from the fruit and then concentrated in evaporators under reduced pressure and briefly exposed to elevated temperatures. During this operation, water and many of the volatile components of orange juice are removed. Unfortunately, many of these volatile compounds contribute to the fresh flavor and aroma of the juice. Thus, the concentration process of orange juice alters its flavor. The addition of fresh juice and concentrated essence to the finished concentrate partially restores its original qualities. Theoretically, to obtain this fresh flavor in the processed juice, those compounds which influence its flavor should be present in the processed product in the same quantities as in the fresh juice.

During the 1960's, primarily through the use of GLC, NMR, MS, and IR analysis, more than 200 volatile compounds were isolated and at least 125 were positively identified as components of orange juice. With the identification process well underway, the next step was the establishment of those contributing significantly to the flavor of the juice, either directly or through synergistic reactions. A step toward this identification was to determine the odor and flavor thresholds of those compounds present.

Orange juice contains aldehydes and nucleotides in relatively

high concentrations. As a class of compounds, aldehydes have long been noted for their flavor and aroma characteristics. Nucleotides have recently received recognition as flavor enhancers (Kuninaka, 1960). A number of studies have been conducted to determine the flavor enhancing potential of nucleotides on various foods; however, little has been published concerning this enhancing potential on individual compounds possessing strong flavors, such as most aldehydes.

Research Plan: The proposed research will necessarily begin with preliminary evaluations of the known volatile constituents of citrus juices, essences, and oils for potential contributors to acceptable citrus flavors. Suggested possibilities are limonene, l-carvone, trans-carveol, valencene, n-undecanol, linalool, octanol, α-terpineol, α-pinene, ethyl butyrate, n-decanol, p-cymene, n-dodecanol, caryophyllene, citrinellol, α-copaene, β-elemene, 1, 8-p-methadiene-9-ol, 1, 8-p-methene-1, 2-diol, n-nonanol, perillyl aldehyde, piperitenone, sabinene, nootkatone, β-sinensal, neral, geranial, nerol, several aliphatic aldehydes, as well as other compounds. The compounds will be purchased commercially, synthesized commercially, or obtained from the Fruit and Vegetable Products Laboratory, U.S.D.A., Winter Haven, Florida, and will be evaluated for purity primarily by gas chromatographic procedures. Contaminated compounds will be purified by pertinent chromatographic means before presentation for flavor panel evaluations.

The flavor panels will involve odor and taste analyses in the form of sniffing and taste tests from odorless containers. Samples will be presented in aqueous media, the condition under which the flavor components are usually encountered in juices. All compounds used in these tests will be those found naturally in citrus juices and oils. Taste panel members will be allowed to taste samples without swallowing if

so desired.

Phase 1. Panel member selection. Triangular tests of juice dilutions as well as individual compounds will be utilized in selection of panel members. Each panel will consist of approximately 20 screened and trained people. Sensitivity and repeatability of the individuals will be the primary criteria for selection of the panelists.

Phase 2. Threshold level determinations. The initial evaluations will be odor and flavor threshold determinations. All samples will be tested in a suitable medium using ranges about or below amounts known or expected in juice as consumed. Threshold levels of several compounds, including several aliphatic aldehydes, esters, and nootkatone, have already been reported. These investigations will be taken into account during the proposed research.

Phase 3. Individual component evaluations. After the lowest detectable levels of the selected potential flavor components are determined, those compounds which could reasonably be contributors to flavor will be further evaluated in a suitable medium. Multiple comparison tests of several levels of individual compounds will be rated on a hedonic scale and compared to a reference citrus juice. These tests should enable the investigators to be even more selective of the compounds contributing to the chosen fresh citrus flavor.

Phase 4. Multiple component evaluations. Further multiple comparison tests of combinations of the compounds found to be most likely contributors to flavor will then be carried out. Again a reference juice will be used for comparison. This reference will consist of high quality commercial concentrate containing essence.

Phase 5. Evaluation of component mixtures in a juice medium. After further selections of the most likely combinations, intensificaitons of

these mixtures by other previously rejected compounds will be evaluated by multiple comparison tests. The final measure of contributions of the selected combinations of compounds to flavor by laboratory panels will be by fortification of juices previously stripped of their volatile components. Such juices will be reconstituted prior to fortification and will be compared with the reference juice. The feasibility of consumer preference taste panels will depend entirely upon the results obtained and developments within the Food and Drug Administration concerning compounds on the GRAS (generally accepted as safe) list.

It should be noted that throughout the proposed research described above, the number of working compounds will become smaller as investigations progress. Such appears to be the most reasonable and orderly manner to keep the scope of the project feasible within the suggested time limits.

All experiments will be designed for evaluations by appropriate statistical analyses.

REVIEW OF LITERATURE

Sensory Evaluation

Flavor has been defined as the complex of sensations through which the presence and identity of foods and beverages in the mouth were determined (Hodges, 1962). Flavor, therefore, is not a single entity, but a complex of effects perceived by several senses. Taste, odor, touch, sight, and hearing are all involved with odor and taste being the principal contributors.

Odor

Sagarin (1954) defined odor as the property of a substance that

has been perceived by inhalation into the nasal or oral cavity and has made an impression upon the olfactory area of the body. This area consists of two patches of yellowish tissue located in its upper portion. Two types of nerve fibers whose endings receive and detect odorous molecules are imbedded in the tissue. The more abundant of these two types of fibers has an olfactory cell bearing a cluster of hair-like filaments that act as receptors. When the olfactory cell is stimulated by an odorous molecule, a signal is sent to the olfactory bulb located just above the olfactory area. From there, the signal is transmitted to higher brain centers (Amoore et al., 1964).

Most workers in this field have postulated that the molecule-receptor interaction is a physical rather than a chemical process. At least three factors are generally thought to contribute to the odor quality of a molecule: size and shape, functional groups, and orientation with respect to the receptor surface (Klopping, 1971). Several theories pertaining to odorant-receptor interaction have been proposed. Of these, Amoore's sterochemical theory (Amoore, 1963) has perhaps been the most widely publicized. Amoore stated that odor was based on the geometry of molecules. He hypothesized that seven different olfactory receptor sites existed, each with a specific geometric shape. Seven primary odors have been postulated: musky, floral, camphoraceous, minty, ethereal, pungent and putrid. Theoretically, a good match between receptor site and compound shape will yield a primary odor response. On the other hand, the less perfect the fit, the more blending of primary odors will occur.

Klopping (1971) reported that a material must meet certain requirements to be odorous: (1) the material must be soluble in organic solvents as well as slightly soluble in water; (2) the material must have

some volatility and be present in the air surrounding the receptor site in a certain minimum concentration which varies greatly between different materials; and (3) there must be direct physical contact between the molecule and receptor site. However, up to this time with the limited neurophysiological knowledge, it has been impossible to predict the odor sensation produced by molecules by just knowing various physical and chemical properties of the odorant (Laffort, 1963).

Taste

Beidler (1966) described taste as that sensation produced when food was taken into the mouth and caused the stimulation of taste bud receptors. The organs of taste are clusters of receptor cells located in the taste bud. Each bud has fingerlike projections, taste microvilli, which extend into the saliva coating of the tongue where contact is made with various components of food. Beidler has theorized that these components are absorbed to specific sites on the microvilli, slightly changing the spatial arrangement of the molecules making up the sufface of the receptor. In some unknown manner, the influence of the filled sites is projected further down the surface of the receptor cell, where minute holes are created in a thin (75Å) lipid-protein cell membrane. Sodium and potassium ions leak through these holes and exchange with similar ions in a thin layer of solution encircling the body of the receptor cell. This stimulates the nerve endings that are closely encircled by the receptor cell membrane. The nerve then develops a series of brief electrical impulses that are carried to the brain, which, in turn, acts as a computer to determine the taste. Little is known concerning the relationship between molecular structure and taste sensation. Although many details concerning the shape and functional properties of the stimulatory molecule are known, similar

Properties of the receptor site with which it reacts are unknown.

Factors Influencing Flavor and Odor

The human subject is not capable of machine-like performances, but is susceptible to influence by many physical and psychological conditions. Perhaps the most important factor affecting one's ability to smell or taste is heredity. Snyder (1931) tested 440 individuals and found that 31.5% were unable to taste p-ethoxyphenylcarbamide due to an inherited taste deficiency. Fisher and Griffen (1963) showed that persons who were sensitive to the taste of phenylthiourea were also more sensitive to the tastes of 33 other compounds than persons insensitive to its taste. One's health also influences his ability to detect flavors. Amerine et al. (1965) stated that at the onset of a head cold, one's olfactory sensitivity increased before it was destroyed by mucous accumulation. Thus, a panelist with a head cold or sinus congestion may suffer a partial or complete loss of olfactory and taste perception after the onset of the congestion.

Blakeslee and Salmon's (1931) results suggested that females were somewhat more acute tasters than males; however, King (1937) showed conflicting results with respect to sex. Using a panel of 35 women and 29 men, he found no statistical differences in their abilities to detect salty, sweet, sour, or bitter tastes. However, by elimination of the least consistent and least discriminating judges, he reduced this group from 64 to 14 judges, 11 of whom were women. Minich et al. (1966) reported that males consistently displayed greater taste sensitivity than did females in determining taste thresholds for standard chicken broth. This was particularly true with respect to young males (19-30) as opposed to young females of the same age group. Amoore and Venstrom (1966) found no convincing evidence of a difference between group thresholds

computed for men and women for the seven primary odors. In this same experiment, no significant differences were obtained when group thresholds were computed for smokers and non-smokers. King (1937) determined that smoking had no effect on individual taste thresholds for sweet (sucrose), salt (sodium chloride), bitter (caffeine), or sour (lactic acid) tastes.

A person's age may or may not affect his ability to taste or smell. Baten (1950) reported that younger panelists (18-20) were more capable of distinguishing differences in strengths of salty and sweet materials than were middle-aged panelists (35-40). This was not true with sour tastes, but the young panelists were more consistent in their decisions pertaining to sour tastes. Minich (1966) stated that while older panelists (34-69) recognized a specific flavor at a lower concentration than younger panelists (19-30), the young group distinguished between two concentrations and recognized differences in flavor intensity better than the older group. King (1937) determined that there were no significant differences in the taste thresholds between panelists under 30 years of age and those over 30 for sweet, salt, and bitter tastes. However, the panelists under 30 years of age had a lower acid (sour) threshold.

Hodges (1962) determined that the intensity of light in the panel room did not affect absolute threshold as much as the absence or presence of light. Absolute thresholds were lower when determined in total darkness. Increased noise level raised the absolute threshold for sweet taste (sucrose), lowered the threshold for sour taste (tartaric acid), and had no effect on salty taste (sodium chloride). Mitchell (1957 a) reported that panelists demonstrated greatest sensitivity when individually tested in a silent environment. It appeared that the knowledge of

the presence of other panelists during a test provided enough disruption to a panelist's concentration to significantly lower his sensitivity. In addition, if there were disruptions during the test, this further decreased a panelist's sensitivity. Mitchell (1957b) determined that Tuesday was the best day of the week for conduction of a taste panel, followed by Friday, Monday, and Thursday, respectively. He also determined that panelists were most sensitive between 11:00 a.m. and 12:30 p.m., followed closely by 1:30 p.m. to 2:30 p.m., during each day of the week. Mitchell concluded that while eating per se may have dulled the sensory receptiveness, other factors counteracted this to the extent of making the period immediately after lunch better than most of the other times of the day. Yensen (1958) also mentioned systematic variations during the day in taste sensitivity. He determined that forced loss of salt from the body was accompanied by an increase in taste sensitivity to salt, while sensitivity to sweet, sour, and bitter tastes were not affected by this factor.

Such factors as sample temperature, coding, color, method of presentation, and environmental conditions of the panel room (temperature and humidity) influenced the panelists' sensitivity (Amerine et al., 1965).

Threshold Theory

Absolute flavor and odor thresholds have been defined in a number of ways, and there are as many methods for determining them as there are definitions. According to Gregson (1962), the number of definitions and methods coupled with individual threshold differences and experimental error have resulted in a wide range of reported absolute thresholds. Swets (1961) doubted the existence of sensory thresholds, while Gregson (1962) stated that there were as many or as few thresholds as one could conceptulize. He gave examples of three types of thresholds: (1) the

threshold of knowing the substance was not water; (2) the threshold of recognizing the substance; and (3) the threshold of recognizing the intensity of the substance. There have generally been two widely used definitions for thresholds. Berg et al. (1955) and Harrison and Elder (1950) defined absolute threshold as that concentration of flavor or odor which was detected 50% of the time from a reference containing pure water. The second definition, or difference threshold, uses a given concentration as the reference. In both cases, a difference is determined between the reference and the given concentration of the compound. Harrison and Elder (1950) and Gregson (1962) stated that flavor perception varied with the log of the concentration of the stimulus (Weber-Fichner Law) and the distribution of scores about the threshold presumably followed the normal probability function. Patton and Josephson (1957) concluded that while there was a linear relationship between detection and log of concentration close to the threshold value, the true relationship taken over all concentrations was a sigmoid relationship. Amerine et al. (1965) also confirmed this sigmoid relationship between detection and concentration.

Panel Methodology

Laffort (1963) stated that large discrepencies in threshold values occurred between authors for the same molecules, essentially due to techniques and conditions used by each one. Schutz (1971) warned that chosen panelists could be unrepresentative of the sensitivity in the population; therefore, even though they were used as an instrument, they would be much more sensitive or insensitive than the population. Thresholds should be considered approximate as established by a particular panel. In order for the panel to be more nearly representative of the general public, a large unscreened and untrained panel is best (Kramer et al., 1961). The desired precision is achieved through the size of

the panel, rather than by a large number of repetitions. However, Kramer et al. (1961) stated that the most efficient panel for determining differences was a small number of well-trained panelists. The desired precision would be achieved through repetitions. As the number of replicate testings increased, there was little, if any, improvement in the efficiency of a trained panel, but considerable improvement in the untrained panel.

Harrison and Elder (1950) presented panelists with a series of paired samples. Each pair contained one dilution of the compound under investigation and one blank. This was known as the series, or multiple paired comparison test. Amerine et al. (1965) stated that the true paired comparison test was the comparison of two samples. The panelist was presented with two samples and asked to indicate if there was a difference between them. In a directional paired comparison test, the panelist must determine which of the two has the greater intensity. This procedure has generally minimized the memory effect associated with other tests. However, if the time interval has not been controlled in paired comparison tests of tastes and odors, there may be an increase in the adaptation effect, carry-over effect, or fatigue. Berg et al. (1955) used the triangle test in determining thresholds. Kramer et al. (1961) determined that a comparison test was several times as efficient in detecting flavor differences as the triangle test.

Schutz (1971) stated that fatigue and adaptation effects could produce serious internal validity problems in one's results. Lane et al. (1954) defined fatigue in organoleptic testing as the diminishing ability of a panelist to recognize small differences in flavor or odor as testing was prolonged. A majority of all researchers recognize the existence of fatigue and provide testing conditions designed to minimize its

effect, thus maintaining the panel at maximum efficiency. Lane et al. performed several experiments which demonstrated the existence of fatigue. They also noted that while fatigue was prominent in the testing of some types of food, it was practically negligible in others. As the difference between two samples decreased, the fatigue and carry-over factors increased. Gregson (1962) implied that fatigue may have been responsible for panelists' indicating a water blank as having taste when placed between two concentrations above the threshold value.

In designing an organoleptic experiment to minimize fatigue, the panelists should be presented one sample per session (Kramer et al., 1961). However, this would be a highly inefficient panel. Kramer remarked that the most confusion and difference of opinion in panel methodology centered around the number of samples presented to a panelist at one sitting. The optimum number presented undoubtedly depends upon the nature of the substance being tested. Nine samples per sitting was found to be most efficient, particularly for mild-flavored products. When a trained panel was used, as many as 18 samples were presented with little effect on efficiency. Tompkins and Pratt's (1959) data indicated that judges could discriminate just as well with seven samples of frozen orange juice as with three samples, with no apparent fatigue. There was greater efficiency with seven samples. In an attempt to minimize fatigue and carry-over, Minich et al. (1966) presented each panelist with seven numbered smaples on a tray in order of increasing concentrations. Gregson (1962) termed this the ascending series method and added that each concentration should be at least twice the strength of the preceding one. Thus, the sum of all the preceding concentrations was never greater than the next concentration tested. Theoretically, if fatigue was cumulative, it did not completely mask the next concentration.

Since a linear relationship usually exists between panel detection and the log of the concentration, linear regression has usually been employed in analyzing experimental results (Harrison and Elder, 1950; Patton and Josephson, 1957; Stone et al., 1962; Meijboom, 1964; Amerine et al., 1965).

Flavor Significance of Orange Juice Components

Volatile Components

The ultimate purpose in identifying the volatile components in orange juice is to attempt to relate them to flavor quality. Patton and Josephson (1957) reported that the flavor significance of an individual compound may be determined by finding the concentration of the compound in orange juice and comparing it to the threshold value in pure water. If the compound was present in excess of its threshold, it probably had a direct effect on flavor. This did not take into account the interacting effects from other compounds which naturally occur in food products.

A need still exists for more information concerning the concentration of the volatile compounds in juice. The volatile content varies with variety, maturity, and physical condition of the fruit (Wolford et al., 1969). The quantitative chemical composition of the essence also tends to vary with the method of essence recovery system being used (Wolford et al., 1969).

The quantity of water soluble volatiles in freshly extracted Valencia orange juice ranged from 374 to 510 ppm (Wolford et al., 1969). Maximum values for Valencia juice have been as high as 1000 ppm, depending upon seasonal variation (Wolford et al., 1969). Generally, lower values occur at earlier fruit maturity. Further analyses of commercial orange essence were made in thirteen processing plants during the 1967-68 season and indicated that ester concentrations

in orange juice ranged from 42 to 119 ppm.

The essential oils are generally divided into hydrocarbons and oxygenated compounds (Bravermann, 1949). In orange oil, the hydrocarbon portion consists mainly of d-limonene, which makes up about 90% of the oil. The oxygenated compounds are considered by Bravermann (1949) to be more important with regard to aroma. These consist of alcohols, aldehydes, ketones, acids, and esters. Wolford et al. (1971) reported that alcohols accounted for about 31.9% of the oxygenated components of orange oil, while esters made up about 21.9%.

Alcohols, Esters and d-Limonene

The specific concentrations of the alcohols, esters, and d-limonene reported in the literature are sketchy and inconsistent. The reported values are difficult to assess since some are given in relation to orange juice, while others are reported in relation to certain fractions of the essence. Kirchner and Miller (1957) reported the concentrations of several volatile alcohols and esters and d-limonene (Table 1). These are given in relation to orange juice. Coleman et al. (1969) reported the percentages of many alcohols, esters, and d-limonene in essence oil (Table 2). The total essence oil in the original orange juice was not calculated in this study. Shaw and Coleman (1971) calculated the percent composition by weight for volatile esters, alcohols, and d-limonene in essence oil (Table 3). Furthermore, there still exists a need for more comparable results for those volatiles that have been previously calculated.

Also involved in the determination of flavor significance was the establishment of threshold values for each compound (Patton and Josephson, 1957). The values published for flavor and odor thresholds suffer from lack of reproducibility. Variations in threshold values are

Table 1. Concentrations of volatile alcohols, esters and di-limonene in fresh orange juice.[a]

Compound	Concentration (ppm)
Linalool	0.93
α-Terpineol	0.32
n-Hexan-1-ol	0.10
n-Octan-1-ol	0.21
n-Decan-1-ol	0.10
3-Hexen-1-ol	0.10
$C_7H_{16}O_2$	0.07
$C_{15}H_{26}O$ (I)	0.07
$C_{15}H_{26}O$ (II)	0.14
Ethyl isovolerate	0.01
Ethyl $C_6H_8O_2$	0.03
Methyl alpha-ethyl-n-caproate	0.06
Citronellyl acetate	0.10
Terpinyl acetate	0.08
d-Limonene	80.1
Total oil	91.6

[a]Kirchner and Miller (1957)

Table 2. Percentages of alcohols, esters and d-limonene Valencia orange essence oil.[a]

Compound	Percentage
n-Octanol	.005
n-Decanol	.01
n-Dodecanol	.025
Nonanol	.007
n-Undecanol	.2
α-Terpineol	-
Linalool	.06
trans-Carveol	4.9
Citronellol	-
1,8-p-Menthadiene-9-ol	.001
1,8-p-Menthene-1,2-diol	-
Ethyl butyrate	.0004
d-Limonene	84.72

[a]Coleman et al. (1969)

Table 3. Percentages of alcohols, esters and di-limonene present in Valenica Orange oil.[a]

Compound	Percentage
Ethanol	2.474
Linalool	.104
trans-Carveol	.374
cis-Carveol	.165
trans-2,8-p-Menthadien-1-01	-
cis-2,8-p-Menthadien-1-ol	-
Ethyl butyrate	2.874
Methyl butyrate	.202
Ethyl propionate	.035
Ethyl acetate	.122
d-Limonene	85.821

[a]Shaw and Coleman (1971)

mainly due to differences in methodology (Pangborn, 1960).

Table 4 includes the published information concerning the threshold values for many volatile alcohols and d-limonene. Table 5 contains the information pertaining to the threshold values of esters. These thresholds were all determined in distilled water. A few of these compounds have been previously determined in various other media, including milk and butteroil (Day et al., 1963, and Siek et al., 1969).

Data for calculations of flavor thresholds (Tables 4 and 5) by Keith and Powers (1968) were collected by presenting the panelists with concentrations of the compound in fruit nectar. If the compound was detectable at a given level, the concentration was decreased until it was no longer detectable. The threshold level was then reported as the concentration where a significant number of tasters could detect the compound, whereas the next lowest level was non-detectable.

Siek et al., (1969) determined the flavor thresholds of several esters (Table 5) by presenting the judges with two reference samples, zero concentration and a very concentrated sample. The judges compared the references to a random series of dilutions, reporting those that were similar to the compound in the highest concentration. The threshold was the concentration that was detected 50% of the time.

Buttery et al. (1971) evaluated the odor thresholds of several alcohols (Table 4) by directly spraying the compound into the nasal cavity. The threshold value was determined by a paired comparison test at the concentration that corresponded to the 0.01 probability level of positive response. This same method was used by Flath et al. (1967).

Aldehydes

In contrast to many other fruits, in which the volatile flavoring

Table 4. Threshold values for volatile alcohols and d-limonene previously reported.

Compound	Threshold (ppb)
Ethanol[a]	100,000
Propanol[a]	9,000
Butanol[a]	500
Hexanol[a]	500
Butanol[b]	500
3-Methylbutanol[b]	250
Hexanol[b]	500
Pent-1-en-3-ol[b]	400
Hex-cis-3-enol[b]	70
2-Methylmercaptoethol[b]	120
Guaiacol[b]	3
Eugenol[b]	6
Linalool[b]	6
α-Terpineol[b]	350
Ethanol[c]	53,300
Maltol[c]	7,100
Benzyl alcohol[c]	5,500
d-Linomene[b]	10

[a]Flath et al. (1967) odor thresholds
[b]Buttery et al. (1971) odor thresholds
[c]Keith and Powers. (1968) taste thresholds

Table 5. Threshold values for volatile esters previously reported.

Compounds	Thresholds (ppb)
Ethyl acetate[a]	5,000
Propyl acetate[a]	57
Butyl acetate[a]	66
2-Methylbutyl acetate[a]	5
Propyl butyrate[a]	18
Butyl propionate[a]	25
Ethyl butyrate[a]	1
Ethyl 2-methyl butyrate[a]	0.1
Ethyl Pentonate[a]	5
Pentyl acetate[a]	5
Hexyl acetate[a]	2
Ethyl acetate[b]	6,600
Ethyl butyrate[b]	15
Amyl valerate[b]	4,700
Ethyl acetate[c]	3,000
Amyl butyrate[c]	1,300
Ethyl caprylate[c]	720
Ethyl acetoacetate[c]	520
Ethyl butyrate[c]	450
Ethyl heptylate[c]	170
Ethyl valerate[c]	94
Ethyl cinnamate[c]	16

[a]Flath et al. (1967) odor thresholds
[b]Siek et al. (1969) taste thresholds
[c]Keith and Powers (1968) taste thresholds

constituents are mainly alaphatic esters, citrus fruits owe their characteristic aroma and flavor to essential oils, of which aldehydes are the principal flavor and aroma constituents (Kefford, 1959). Not only is the aldehyde content indicative of the flavoring qualities, but the quality of orange essence is based upon its aldehyde content (Kesterson and Hendrickson, 1953). Orange essence, a by-product of the concentration process, was composed primarily of hydrocarbons with lesser amounts of oxygenated compounds. Peel oil, that oil contained within the flavedo, has been obtained either mechanically or extracted with organic solvents. This oil also contained hydrocarbons and oxygenated organic compounds. Kirchner and Miller (1957) and Kirchner (1961) stated that some peel oil will be incorporated into the juice during extraction. Significant amounts of oil are also contained in the juice sacs, and it is the oil within the juice sacs that gives the typical flavor and aroma. This oil is volatile and relatively water insoluble. Wolford and Attaway (1967) determined that oxygenated compounds contributed significantly to the flavor and originated in the juice vesicles. Bruemmer (1969) demonstrated that juice vesicles contained coenzymes and enzymes to carry out oxidation and reduction, and that these reactions could take place within the vesicle. He also stated that some of the reactions undoubtedly are involved in the formation of specific flavor compounds.

The yield of oil in fresh orange juice depends upon several factors: method of juice extraction, variety and maturity of the orange, handling and storage after picking, tree location and environment, and climatic conditions (Kirchner, 1961; Lifshitz et al., 1970). Rice et al. (1952) extracted the juice from Valencia oranges, being careful not to contaminate the juice with peel oil. They determined that the juice contained 0.004 - 0.006% oil. Blair et al. (1952), using a different

extraction procedure, determined that the juice contained about 0.005% oil. The geometric mean of the two reported results was about 0.001%, or 10,000 ppb. Kesterson and Hendrickson (1953) determined that the aldehyde fraction of Valencia oil ranged between 1.38 and 2.02%, with a mean of about 1.70%. Lifshitz et al. (1970) reported an average of 1.53%, and Petrus et al. (1970) reported a range of 1.69 to 3.17%, with a mean of 2.43%. Thus, the concentration of aldehydes in fresh Valencia juice is approximately 170 ppb. Previously, Naves (1947) had determined that about 31% of the aldehydes found in the juice was octanal. Thus, the concentration of octanal would appear to be about 53 ppb. Kirchner and Miller (1957), in an extensive study of the volatile oil constituents of Valencia juice, determined that the total aldehyde concentration was about 250 ppb and the concentration of octanal was 60 ppb.

In the early analyses of the volatile constituents of orange juice, only ethanal was isolated and identified (Hall and Wilson, 1925). Naves (1947) identified several aldehydes by comparing chemical and physical properties of the unknown semicarbazones to those of known aldehydes. More recently, a combination of vacuum distillation, DNPH derivatives, and TLC have been used to isolate and identify aldehydes (Kirchner and Miller, 1957).

Since the advent of GLC in the late 1950's, more than 200 volatile constituents have been isolated (Attaway et al., 1962; Wolford et al., 1962; Wolford, 1963; Wolford and Attaway, 1967; Coleman et al., 1969; Coleman and Shaw, 1970; Lund et al., 1971). In some cases, aldehydes were determined both qualitatively and quantitatively. Bernhard (1960) attempted one of the initial separations of essential oil components using GLC. During the last ten years, improvements in Bernhard's methods have been made and many new techniques implemented (Stanley et al., 1961; Wolford et al., 1962; Schultz et al., 1964; Wolford and Attaway, 1967;

Coleman and Shaw, 1971; and Dinsmore and Negy, 1971). These researchers used combinations of the following steps to isolate and identify aldehydes in orange juice: (1) gross separation of essence oil from the juice by utilizing various vacuum distillation methods; (2) separation of oil into individual components by GLC; (3) identification of individual components by classical and/or spectral methods. The aldehydes which have been positively identified as natural components of Valencia orange juice are listed in Table 6. Tentatively identified compounds have not been listed.

Thresholds

Lea and Swoboda (1958) used a large, unscreened and untrained panel to determine flavor thresholds so that the panel precision would more nearly represent the population. To prevent the panel from becoming too familiar with each aldehyde, each series of samples was submitted only twice. They defined flavor threshold as the level at which the aldehyde could be detected by 50% of the panelists. Thresholds were determined by plots of the percentage of panelists detecting the aldehyde versus the log of the concentration. They noted a considerable variation in the sensitivity between individual members of the panel.

Guadagni et al. (1963b) used a small, selected and trained panel for determining odor thresholds. Thresholds were determined by evaluating two or more series of three to six concentrations each. Each concentration was paired with samples of triple-distilled, odor-free water, and the panelists were asked to check the sample that contained the odorous material. Position of the water was randomized in each pair, and the presentation of concentrations was also randomized. The percent of correct responses was plotted against concentration. However, he defined the threshold as that concentration which gave the percent of correct responses corresponding to 99% probability level. Because of the insolubility of some aldehydes, these aldehydes were first dissolved in ethanol, which has a relatively high odor and flavor threshold.

Table 6. Aldehydes established as components of fresh orange juice.

Aldehyde I.U.C. Name (common name)	Method of Isolation-Identification	Quantitative Date (concentration in fresh juice	Reference Reporting Identification
benzenecarbonal (benzaldehyde)	GLC - MS		Schultz et al. (1967)
butanal (butylaldehyde)	GLC - MS		Schultz et al. (1964)
	N_2 - GLC, MS		Dinsmore and Negy (1971)
citral a (geranial)	GLC - classical		Attaway et al. (1962)
	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
	GLC - MS		Schultz et al. (1967)
	GLC - PE		Wolford and Attaway (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - classical	17ppb	Lifshitz et al. (1970)
	GLC - MS, IR	10 - 15ppb	Coleman and Shaw (1970)
citral b (necal)	GLC - classical		Attaway et al. (1962)
	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
	GLC - MS		Schultz et al. (1967)
	GLC - PE		Wolford and Attaway (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - classical	approx 2ppb	Lifshitz et al. (1970)
	GLC - MS, IR		Coleman and Shaw (1970)
citral a & b	distillation-classical	14ppb	Naves (1947)
	GLC - classical		Bernhard (1961)
	GLC - MS		Schultz et al. (1964)
	GLC - MS		Teranischi et al. (1966)

Table 6 continued

citronellal (d-rhodinal)	CNPH,TLC - classical	40ppb	Kirchner and Miller (1957)
	GLC - classical		Bernhard (1961)
	GLC - classical		Wolford et al. (1963)
	GLC - MS		Schultz et al. (1967)
	GLC - PE		Wolford and Attaway (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
decanal (capraldehyde)	distillation-classical	46ppb	Naves (1947)
	DNPH, TLC - classical	50ppb	Kirchner and Miller (1957)
	GLC - classical		Bernhard (1961)
	GLC - classical	72ppb	Stanley et al. (1961)
	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
	GLC - MS		Schultz et al. (1964)
	GLC - MS		Teranishi et al. (1966)
	GLC - MS		Schultz et al. (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - classical	70ppb	Lifshitz et al. (1970)
	GLC - MS, IR	47-70ppb	Coleman and Shaw (1971)
dodecanal (lauraldehyde)	distillation-classical	10ppb	Naves (1947)
	GLC - classical		Bernhard (1961)
	GLC - classical	7.9ppb	Stanley et al. (1961)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - classical	8.5ppb	Lifshitz et al. (1970)
	GLC - MS, IR		Coleman and Shaw (1971)
ethanal (acetaldehyde)	distillation-classical		Hall and Wilson (1925)
	DNPH, TLC - classical	3,000ppb	Kirchner and Miller (1957)
	GLC - classical		Attaway et al. (1962)
	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
	GLC - MS		Schultz et al. (1964)
	GLC - MS		Schultz et al. (1967)
	GLC - PE		Wolford and Attaway (1967)
	N_2 - GLC, MS		Dinsmore and Negy (1971)

Table 6 continued

furfural (fural)	GLC - classical		Bernhard (1961)
	N_2 - GLC, MS		Dinsmore and Negy (1971)
hendecanal (N-undecanal)	GLC - classical	3.1ppb	Stanley et al. (1961)
	GLC - classical		Bernhard (1961)
	GLC - classical		Wolford et al (1963)
	GLC - PE		Wolford and Attaway (1967)
	GLC - classical	1ppb	Lifshitz et al. (1970)
heptanal (enanthaldehyde)	GLC - classical	4.4ppb	Stanley et al. (1961)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - IR		Coleman and Shaw (1971)
hexanal (caproaldehyde)	DNPH, TCL-classical	40ppb	Kirchner and Miller (1957)
	GLC - classical		Attaway et al. (1962)
	GLC - classical		Wolford et al. (1962)
	GLC - MS		Schultz et al. (1964)
	GLC - MS		Teranishi et al. (1966)
	GLC - PE		Wolford and Attaway (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - MS		Coleman and Shaw (1971)
2-hexenal, cis	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
2-hexenal, trans	GLC - classical		Attaway et al. (1962)
	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
	GLC - MS		Schultz et al. (1967)
	GLC - PE		Wolford and Attaway (1967)
nonanal (pelargonaldehyde)	distillation-classical		Naves (1947)
	GLC - classical		Bernhard (1961)
	GLC - classical	9.2ppb	Stanley et al. (1961)
	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
	GLC - MS, IR, NMR		Teranishi et al. (1963)

Table 6 continued

	GLC - MS		Teranishi et al. (1966)
	GLC - PE		Wolford and Attaway (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - classical	10.2ppb	Lifshitz et al. (1970)
	GLC - MS, IR	10ppb	Coleman and Shaw (1971)
octanal (capryl aldehyde)	scraping-classical	110-130ppb	Benezet and Igolen (1946)
	distillation-classical	40-80ppb	Naves (1947)
	DNPH - classical	60ppb	Kirchner and Miller (1957)
	GLC - classical	66ppb	Bernhard (1961)
	GLC - classical		Stanley et al. (1961)
	GLC - classical		Attaway et al. (1962)
	GLC - classical		Wolford et al. (1962)
	GLC - classical		Wolford et al. (1963)
	GLC - MS, IR, NMR		Teranishi et al. (1963)
	GLC - MS		Teranishi et al. (1966)
	GLC - MS		Schultz et al. (1967)
	GLC - PE		Wolford and Attaway (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - classical	60ppb	Lifshitz et al. (1970)
	GLC - MS, IR	47-89ppb	Coleman and Shaw (1971)
2-octenal (cis and trans)	GLC - PE		Wolford and Attaway (1967)
perillaldehyde (perylla aldehyde)	GLC - MS		Schultz et al. (1964)
	GLC - MS		Schultz et al. (1967)
	GLC - MS, IR		Moshonas and Lund (1969a)
	GLC - MS, IR	1.5ppb	Coleman et al. (1969)
	GLC - MS, IR	<10ppb	Coleman and Shaw (1971)
sinensal, α -	GLC - MS, IR		Moshonas and Lund (1969a)
sinensal, β -	GLC - MS, IR		Moshonas and Lund (1969b)

Flath et al. (1967) reported an odor threshold for ethanol of 100,000 ppb, while Berg et al. (1955) reported a flavor threshold of 40,000 - 52,000 ppb. Keith and Powers (1968) confirmed the flavor threshold of ethanol at 52,000 ppb. Table 7 lists the flavor and odor threshold values for aldehydes.

Forss (1969) reported that the physical nature of the medium in which aldehydes were dispersed affects their flavor threshold. Generally, odor and flavor thresholds for aldehydes were much lower in an aqueous (polar) than in an oily (non-polar) medium (Table 8). Aldehydes with little polarity (essentially non-polar) had much lower flavor and odor thresholds in aqueous media than in non-aqueous media. Meijboom (1964) determined that the taste thresholds for 31 aldehydes were lower in all cases than their corresponding odor thresholds when determined in a non-polar medium. Meijboom also observed that aldehydes with odd numbers of carbon atoms had lower thresholds than aldehydes with even numbers of carbon atoms (Table 8). He commented that this effect appeared in various series of taste and odor experiments, but an explanation of this phenomenon has not been found. Guadagni et al. (1963a) mentioned that aldehydes with higher molecular weights, from C_5 to C_{10}, required fewer molecules to stimulate the olfactory mechanism. Also, branched, chain aldehydes appeared to have much lower thresholds than corresponding straight-chain compounds. In most cases, n-alkanals exhibited lower thresholds than n-alkenals, 2-nonenal being a notable exception with an odor threshold of 0.08 ppb (Guadagni et al. 1963a). Meijboom (1964) reported that the position of the double bond influenced flavor intensity and that trans-isomers of aldehydes in oil media generally had higher thresholds than cis-isomers. Lea and Swoboda (1958) obtained results which indicated that the flavor thresholds for the aldehydes in aqueous solutions decreased from C_3 to C_{12}, and then rose sharply for C_{14}. The most potent members of the series were C_8 through C_{12}. Guadagni et al. (1963a) determined that there existed essentially a linear relationship between the log of the threshold in molecules per ml and chain length.

Table 7. Flavor and odor thresholds of aldehydes determined in aqueous media.

Aldehyde	Odor Threshold (ppb)	Reference Reporting Threshold	Flavor Threshold (ppb)	Reference Reporting Threshold
ethanal	15	c	1500	a
	4	i	--	-
	21	f	--	-
propanal	9.5±1	d	170	g
	170	j	--	-
butanal	70	b	70	g
	9±2	d	--	-
pentanal	12±2	d	70	g
hexanal	5	c	30	g
	4.5±1	d	16±3	h
	30	j	--	-
heptanal	3±1	d	31	h
octanal	.7±.2	d	5	g
nonanal	1±.2	d	--	-
decanal	.1±.04	d	7	g
undecanal	5±2	d	--	-
dodecanal	.2±.06	d	.94	g
	--	-	11	h
tetradecanal	--	-	60	g
trans-2-hexenal	17	c	--	-
	17±4	d	--	-
geranial	32	b	--	-
benzaldehyde	350	b	1500	e
furfural	3,000	b	--	-

[a] Berg et al. (1955)

[b] Buttery et al. (1971)

[c] Flath et al. (1967)

[d] Guadagni et al. (1963a)

[e] Keith and Powers (1968)

[f] Laffort (1963)

[g] Lea and Swoboda (1958)

[h] Sick et al. (1969)

[i] Stahl (1971)

[j] Weurman (1963)

Table 8. Flavor and odor thresholds of aldehydes determined in non-aqueous media.

Aldehyde	Odor Threshold (ppb)[a]	Flavor Threshold (ppb)[a]	Flavor Threshold (ppb)[b]	Flavor Threshold (ppb)[c]	Flavor Threshold (ppb)[d]
Propanal	3,600	1,600	430	200	1,000
Butanal	150	24	190	200	700
Pentanal	240	150	130	-	-
Hexanal	320	150	50	300	600
Heptanal	3,200	42	120	-	-
Octanal	320	68	460	900	600
Nonanal	13,500	320	220	-	-
Decanal	6,700	1,000	240	600	700
Dodecanal	3,000	46	-	900	400
trans-2-Hexenal	10,000	2,500	67	-	-

[a] Meijboom (1964) Thresholds determined in paraffin oil

[b] Day et al. (1963) Thresholds determined in homogenized milk

[c] Lea and Swoboda (1958) Thresholds determined in groundnut oil

[d] Lea and Swoboda (1958) Thresholds determined in paraffin oil

Buttery et al. (1969) determined higher molecular weight homologs of aldehydes up to C_9 were more volatile in dilute water solutions than lower molecular weight homologs. Table 9 lists the air water partition coefficients (weight aldehyde per ml air/weight aldehyde per ml water) for the homologous series of n-alkanals, C_2 through C_{12}, as determined by Buttery et al. (1969).

Meijboom (1964) stated that the addition of aldehydes to one another in paraffin oil produced a masking effect in both taste and odor. However, Guadagni et al. (1963b) reported opposite results in aqueous solutions. They stated that a mixture of unsaturated, saturated and branched-chain aldehydes gave an additive effect and that the additivity of sub-threshold concentrations of aldehydes appeared to be widespread. This could be of importance in determining the characteristic flavor of various food products. In an experiment they conducted, ten saturated aldehydes, each present in one-tenth its threshold concentration, gave an odor. Also, their results showed that octanal had a slight synergistic effect in addition to the additive effect.

Table 9. Air-water partition coefficients for the homologous series of aldehydes at 25°C[a].

Aldehyde	Air-Water Partition Coefficient
ethanal	$2.7 \pm 0.5^{b} \times 10^{-3}$
propanal	$3.0 \pm 0.1 \times 10^{-3}$
butanal	$4.7 \pm 0.3 \times 10^{-3}$
pentanal	$6.0 \pm 0.1 \times 10^{-3}$
hexanal	$8.7 \pm 0.6 \times 10^{-3}$
heptanal	$11 \pm 1 \times 10^{-3}$
octanal	$21 \pm 4 \times 10^{-3}$
nonanal	$30 \pm 4 \times 10^{-3}$

[a]Buttery et al. (1969)

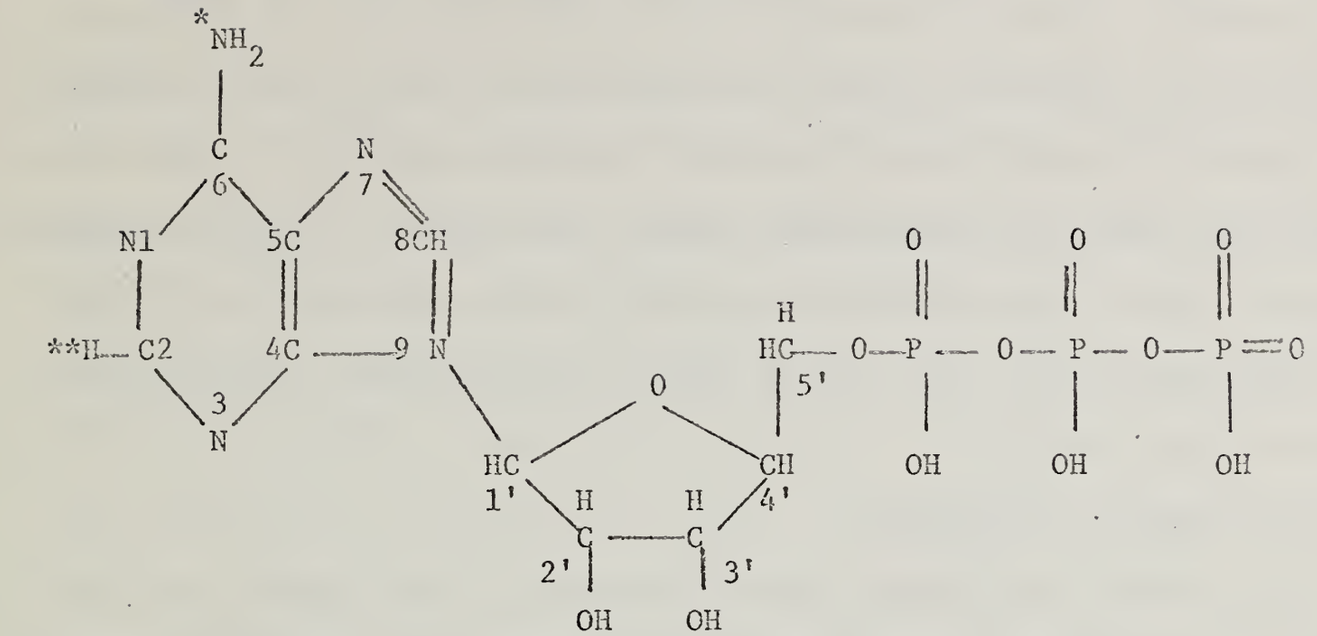
[b]standard deviation

Flavor Enhancement by 5'Nucleotides

Since food flavors are so complex, it is quite possible that food preferences can be increased by inhibiting some components to allow others to be dominant, and increasing flavor components that are natural to the particular food (Beidler, 1966). It is difficult to isolate, identify, and recombine odors to resemble the natural aroma. Thus, we might better turn our attention to enhancing or inhibiting wanted or unwanted odors within a natural product without affecting most of the complex odors that remain. The real value of inhibitors and potentiators lies in the fact that they are specific. Objectionable qualities can be inhibited, or the acceptable qualities accented without changing the properties of a complex aroma (Kuninaka, 1966). Recently, 5'-nucleotides have received much attention as aroma and flavor potentiators (Wagner et al, 1963; Kurtzman and Sjostrom, 1964; Shimazono, 1964; Kuninaka et al. 1964; Shimazono, 1965; Kuninaka, 1966; Hashida et al, 1966; Yamaguchi et al, 1967; Yamaguchi, 1967; Schwimmer and Guadagni, 1967; Stier et al, 1967; Luh and Chen, 1969; Woskow, 1969; Jones, 1969; Yamaguchi, 1971).

Molecular Configuration

Nucleosides are formed by attaching a purine or pyrimidine base to the 1' position of ribose or deoxyribose (Bennett and Freiden, 1966). A nucleotide is the phosphate ester of a nucleoside (Figure 1). If this ester linkage is formed between the phosphate and the 5' carbon of ribose, a 5'-nucleotide is formed. Nucleotides in which the phosphoryl group is attached to a phosphate or a pyrophosphate are diphosphate and triphosphate nucleotides, respectively. By replacing the amino group on the number 6 carbon of the pyrimidine ring of AMP with a hydroxy group, and substituting an H or NH_2 group on the number 2 carbon, the 5'-monophosphate of either IMP or GMP is formed (Wagner et al, 1963). Shimazono (1964) stated that disodium 5'-ribonucleotides are odorless white crystalline powders. They are generally quite soluble in water and acid, but barely soluble in organic solvents. They are chemically stable and do not



PURINE BASE | RIBOSE | PHOSPHATE | PYROPHOSPHATE

ADENINE

ADENOSINE

ADENOSINE 5' -MONOPHOSPHATE

ADENOSINE 5' -DIPHOSPHATE

ADENOSINE 5' -TRIPHOSPHATE

$*NH_2$ = OH — INOSINE 5' -MONOPHOSPHATE

**H = NH_2, *NH = OH — GUANOSINE 5' -MONOPHOSPHATE

Figure 1. General chemical structure of 5' -nucleotides.

decompose when heated to normal cooking temperatures or under usual storage conditions. When ribonucleotides were held one hour in water at 120°C, about 20% decomposed. However, Shimazono (1965) added that when ribonucleotides were heated for one hour at 100°C in an acid medium (pH 3), severe decomposition occurred. One of the most important chemical properties is that certain enzymes (phosphates or phosphomonoesterases) are capable of breaking phosphate linkage of the 5'-ribonucleotides, resulting in the complete loss of flavor enhancing properties. Also, AMP may undergo rapid autolytic deamination to form IMP (Jones, 1969).

Nucleotide Content of Oranges

Barmore (1972) determined that the ribonucleic acid (RNA) content of sweet oranges depended upon the variety and maturity of the orange. The RNA content of pulp for Hamlin, Pineapple, and Parson Brown varieties ranged between 420 and 820, 300 and 1,000, and 100 and 1,000 ppm, respectively. The acid soluble nucleotide content of pulp for the three varieties ranged from 2.0 to 4.0, 2.7 to 4.3, and 1.4 to 4.7 ppm, respectively. Finally, Barmore determined that the ATP content of pulp ranged from 5.1 to 83, 13 to 71, and 5.1 to 65 ppm, respectively. Orange pulp included the juice and juice sacs only.

Non-Volatile Components

Sugar

Sugar is a very important contributor to orange juice quality and greatly influences flavor by imparting sweetness (Tressler et al., 1939). It occurs in juice in greater quantities than any other component except water. At the time of harvest, sugar composes approximately 9 to 10% of Valencia orange juice. This figure is approximated from recent statistics concerning the soluble solids to acid ratio at the time of processing (Florida Citrus Mutual 1969). Sugar has been found to contain 50% sucrose, 24% glucose, and 26% fructose (Tressler et al., 1939). Sugar quality varies according to variety, time of harvest and environmental and cultural factors, including soil, fertilizer, and climatic conditions (Sinclair, 1961).

According to Valdes et al., (1956a) taste panelists ascribed greater flavor to sweeter solutions, but beyond an optimal level, sweetness interfered with flavor perception by decreasing the panelists' ability to discriminate differences. They also suggested that apparent flavor intensity depended on optimal soluble solids and optimal acidity in fruit nectars. Berg et al. (1955a) determined that the taste thresholds for sucrose, glucose, and fructose, respectively, were as follows (ppm): 3100, 4400, and 1300.

Acids

Acid, like sugar, is also an important flavor contributor and imparts a sour taste to orange juice (Tressler et al., 1939). It accounts for approximately 0.8 to 1.0% of the juice (Florida Citrus Mutual, 1969). Acids in orange juice are composed largely of citric acid (approximately 88% of the total acids) and a lesser proportion of malic acid (approximatley 12% of the total acid) (Tressler et al., 1939, and Ting and Attaway, 1971). The acid percentages vary due to environmental conditions and cultural practices (Sinclair, 1961). Very small amounts of other acids appear in orange juice, including oxalic acid, tartaric acid, benzoic acid, succinic acid, and ascorbic acid (Tressler and Joslyn, 1961).

Pangborn (1960) found that the greater the acidity in fruit nectars, the greater the depressing effect on perception additional components. The flavor thresholds for citric and malic acids were reproted to be 23 and 26 ppm, respectively (Berg et al., 1955a).

Pectin

Pectin has not been previously reported to be associated with the flavor of orange juice since it occurs in such small quantities. It is present at about 0.4 g/l of orange juice (Rouse, 1971). Pectin is commonly associated with texture or consistency, and texture is considered to be an important part of flavor (Moncrieff, 1967). Crocker (1945) remarked that a thin liquid would probably taste weaker if a thickening agent were present. Mackey et al. (1956) evaluated the four primary tastes in liquid, foam, and gel media and determined their thresholds. The tastes were easier to detect in the liquid, and most difficult to detect in the gel media.

Interaction of Components in a Mixture

Interactions among taste qualities of food have been a subject of much opinion and speculation. The early literature concerning taste interrelationships in aqueous solutions of pure compounds is very confusing since there were many conflicting conclusions (Pangborn, 1960). There have been several studies dealing with threshold levels and interactions in aqueous solutions of volatile substances (Keith and Powers, 1968).

Most of the early work consisted of measuring the effects of primary tastes on each other. Fabian and Blum (1943) studied the competitive and compensatory actions of the subthreshold levels of one taste on suprathreshold levels of another taste when the two were mixed together. If the subtreshold compound influenced the suprathreshold compound, compensatory action was assumed. If the responses to the suprathreshold compound were not affected, competitive action was assumed. The tests were accomplished by placing a subtrheshold

compound in a solution containing a suprathreshold compound and having the panel compare the solution to other samples containing only the suprathreshold compound. They concluded that citric acid and malic acid did not affect the sweetness of glucose; however, the sweetness of sucrose was increased by citric and malic acid. The sweetness of fructose was reduced by citric acid, but no change was noted in the presence of malic acid. Sucrose reduced sour taste, particularly that of malic acid. It was also corcluded that the data definitely showed true compensatory action of one taste on another.

To clarify some previous contradictions, Pangborn (1960) used then highly trained tasters and found that each compound representing the basic tastes generally depressed the intensity of the others. However, a few had no effect on the compounds. The most pronounced effect was the reduction of the sweetness of sucrose by citric acid and vice versa. Kamen et al (1961) also reviewed interactions in detail. They concluded that sucrose decreased the sourness of citric acid, and that citric acid increased the sweetness of sucrose. The latter conclusion did not agree with the results from Pangborn (1960, 1961). The difference was thought to be due to the difference in methods. Both methods were similar to those used by Fabian and Blum (1943); however, Kamen et al (1961) used a very large number of judges, each judge having been used only once. Pangborn used the same judges in several repetitions.

Pangborn (1961) later confirmed her results in a more detailed study using a paired and single stimulus test. The depressing effect of citric acid on sucrose was greater at lower concentrations than at higher concentrations of sugar. Pangborn (1960) also found that by increasing the sugar and acid content of apricot and pear nectars, there was a decrease in accuracy with which judges could match the taste of a standard to its respective series.

Several studies have shown an organoleptic interaction by testing the threshold of one compound in different solutions. Berg et al. (1955b) made

determinations of thresholds and minimum differences for sucrose at 0, 1, 5, 10, and 15 g/100ml concentration levels in aqueous ethanol-water, acid-water, and acid-ethanol-water solutions. The thresholds and minimum detectable differences were determined using triangle tests. The single effect of sugar concentrations on the minimum detectable differences was highly significant. The single effect of acid was to increase the threshold for sugar significantly. An absence of interaction between the sugar and acid was shown. Hinreiner et al. (1955a) studied the effect of sucrose, acid, alcohol, and glycerol on each other. Sucrose increased both the threshold and detectable difference concentrations for ethanol. The presence of organic acids appeared to diminish the effect of sucrose on alcohol. The single effect of the acid was to increase the threshold for alcohol. Both alcohol and acid increased threshold concentrations for glycerol. The acid threshold was unaffected by sucrose, but was increased by alcohol.

Others found that threshold levels and minimum detectable differences varied according to the solvent. Hinreiner et al. (1955b) found that minimum detectable differences varied for glycerol, tannin, and sulfur dioxide between red and white wines; whereas sucrose, ethyl acetate, and acetaldehyde showed no significant differences. They also found that minimum concentrations for detectable differences were higher in wine than in water. Siek et al. (1969) found similar results when measuring thresholds in water, oil, and butter media with single stimulus tests. Thresholds in oil were higher than in water, and the thresholds in butter media were very close to those in the oil.

Bennett et al. (1965) studied the effect of fat, pH, and volatile acids on the perception of diacetyl in fermented dairy products. Using a paired comparison test, they concluded that the fat in cream probably suppressed the effects of acid and allowed diacetyl to be perceived more easily. The threshold of diacetyl in skim milk increased with decreasing pH; however, small amounts of acid were thought to sharpen the taste for diacetyl. The presence of volatile acids affected the diacetyl differently depending upon the acid. In

general, volatile acids lowered the threshold. Acetic acid and acetaldehyde had such strong effects on diacetyl in sour cream that the threshold could not be accurately established.

Day et al. (1963) found additive interactions between carbonyl components. An additive interaction was exhibited when half the threshold concentrations of two components were mixed together and a detectable taste was noticed. It is interesting to note that when an interaction occurred, the compounds had similar flavor thresholds. Guadagni et al. (1963b) found similar additive effects of subtrhrehold concentrations of compounds associated with food aromas. They concluded that members of a single class of compounds may be expected to exhibit an additive effect, but members of different classes with different structures would not be expected to behave in this manner. Langler and Day (1964) reported that certain ketone mixtures exhibited a synergistic interaction whereby a perceptible flavor became evident when the concentrations of the components in the mixture were in subthreshold levels. Siek et al. (1969) reported similar results among methyl ketones and free fatty acids. In the same study, interactions among aldehydes were weak and interactions among lactones were not apparent.

MATERIALS AND METHODS

For the purposes of this study, flavor is defined as the overall sensual response to a material taken into the mouth, including odor, taste, and other mouth sensations. Odor is that sensation perceived by the olfactory organs and was determined by smelling the samples.

Threshold is defined as that concentration at which 50% of the panelists indicated a difference between a sample containing a given concentration of aldehyde and a reference sample.

Panel Design

The method selected for panel evaluation of samples was the multiple paired comparison test, which has either been employed or recommended by several workers for the same purpose (Harrison and Elder, 1950; Guadagni et al, 1963a; Yamaguchi, 1967; Woskow, 1969). Each pair of samples contained a reference and one dilution of the aldehyde under investigation. Five pairs were presented at each session. This enabled an efficient number of concentrations to be presented per session, but kept fatigue and carry-over effects at a minimum. The concentrations were presented in ascending order, as recommended by Gregson (1962) and Minich et al. (1966), to reduce carry-over effects. Panelists were asked to sample each pair in order and indicate if the samples were alike or different. Panelists were encouraged to take as much time as needed and were permitted to make repeated comparisons within each pair, but could not go back to previous pairs. They were requested not to guess at their answers. If they were not sure of their decision, they were instructed to indicate that the two samples were alike. According to Mitchell (1957a), one advantage to the paired comparison test was that it required the least thought and memory of sensory tests. Thus, the panelists' results might be somewhat automatic, or machine-like.

The testing area was a specifically designed panel room consisting of two separated areas, a preparation area and a sensory testing area. The testing area was a subdivided into eight individual testing booths, each equipped with its own light source, running water, and rotating sample stage for presenting and retrieving samples. Panels were conducted under orange lighting of relatively low intensity. The air temperature and humidity could not be specifically regulated in the panel room, but were similar to that of the entire building, usually 78°F and 50-55%, respectively.

Materials

Citral, decanal, dodecanal, ethanal, hexanal, nonanal, octanal, and trans-2-hexenal were purchased from Aldrich Chemical Company, Inc., (Milwaukee, Wisconsin). Butanal was purchased from Eastman Kodak Company (Rochester, New York). Perillaldehyde was donated by the United States Citrus and Subtropical Products Laboratory (Winter Haven, Florida). The purity of the aldehydes was checked by GLC. All aldehydes were 99+% pure with two exceptions; trans-2-hexenal was approximately 98% pure with the majority of the impurity cis-2-hexenal. Citral was composed of approximately 66% citral a (geranial), and 33% citral b (neral). The aldehydes were kept refrigerated except when used because of their instability. Aldehydes are known for their ability to oxidize, reduce, or undergo aldol condensation reactions with themselves (Morrison and Boyd, 1966; Moshonas and Lund, 1969a). Absolute ethanol was purchased from the United States Industrial Chemicals Company (New York).

The following 5'-nucleotides were purchased from P.L. Biochemicals, Inc. (Milwaukee, Wisconsin): disodium guanosine 5'-monophosphate, sodium guanosine 5'-diphosphate, disodium guanosine 5'-triphosphate, adenosine 5'-monophosphate, disodium adenosine 5'-diphosphate, and disodium adenosine 5'-triphosphate. The nucleotides were kept in a desicator and refrigerated due to the instability of the tri- and diphosphates.

Water used in preparing the various concentrations was distilled twice and

boiled for at least one hour. The boiled water was then placed in a covered carboy and cooled to room temperature for use the following day. Metal containers tended to impart a metallic taste to the distilled water, while plastic equipment imparted a plastic flavor and aroma. Thus, glass equipment and containers were used exclusively for the second distillation, boiling, storage of the boiled water, and during the preparation of all samples presented to the panel for sensory evaluation. The distilled water remained relatively odorless and flavorless for up to 24 hours after boiling, but would acquire an atypical flavor when held much longer.

Two-ounce, wide-mouth, amber glass bottles, Parafilm (American Can Company, Neinak, Wisconsin), and 25-ml pipettes were purchased from Arthur H. Thomas Company (Philadelphia, Pennsylvania). A pipette was used to deliver 25 ml of each concentration into the two-ounce bottles. The bottles were washed using a small amount of detergent in hot water, rinsed twice with distilled water, and dried prior to filling. The bottles were capped using approximately two-inch square pieces of Parafilm. Various plastic screw caps were tested, but all imparted a flavor and/or odor in the distilled water, which the panelists detected. Parafilm was not only odorless and flavorless, but extremely impermeable to water and water vapor.

Determination of Thresholds

Sample Preparation

Ethanal and butanal were soluble in water; however, the remainder of the aldehydes were relatively insoluble. Thus, to keep the procedure uniform, all aldehydes were first dissolved in absolute ethanol, a procedure similar to that employed by Guadagni et al. (1963a). Initially 0.1 mg of aldehyde was diluted by 9.9 ml of ethanol to give a 1:100 dilution. Subsequent dilutions with distilled water were on a volume/volume basis. The reference samples consisted of distilled water and were randomized within each pair. Occasionally, a pair contained two reference samples, that is, two water blanks. All dilutions were

prepared no more than five hours before being presented to the panelists, since most aldehydes in aqueous solutions were relatively volatile.

Testing Procedure

Panelists employed to determine aldehyde flavor and aroma thresholds were volunteers consisting of students and employees of the University of Florida. Occupations included clerks, secretaries, receptionists, printers, and laboratory technicians. There were 52 females and 23 males utilized at various stages of work, ranging in age from 16 to 66 years, and averaging 26.9 years. This large group of panelists was unscreened and untrained, as recommended by Kramer et al. (1961), Meijboom (1964), and Keith and Powers (1968), for determining thresholds better representing those of the population. Twenty-five percent of the panelists were smokers (i.e., more than one cigarette or cigar per week).

Previously reported thresholds were used as a guide in preparing concentrations presented to the panel. When previous trheshold values were not available, an informal panel was held utilizing both regular panelists and other persons to give a gross estimation of a threshold range. These results were not used in determining threshold values. Panel sessions were held Mondays, Wednesdays, and Fridays, from 1:30 to 3:00 P.M.

Odor and flavor threshold concentrations were determined at the rate of approximately two per month for all ten aldehydes. The number of panelists ranged between 25 and 40 for the determination of each threshold. Since the investigation lasted approximately one year, there was some turnover in panel members. Thus, panelists determining the odor threshold of an aldehyde were not necessarily the same as those determining its flavor threshold. In most cases, panels were replicated a sufficient number of times so that a minimum of 100 responses were obtained for each concentration used in determining a particular threshold. To encourage attendance at panel sessions, refreshments were served to panelists upon completion of the sensory testing period.

Statistical Analysis

When a panelist indicated two samples of a given pair were alike, the corresponding concentration received a score of zero. If the two samples were judged to be different, the concentration received a score of one. The scores for each concentration presented were summed for all the panelists and repetitions, divided by the total number of responses, and multiplied by 100 to give the percent of panelists indicating a difference between the concentration and reference. Four concentrations were analyzed to determine each threshold concentration using inverse linear regression, the regression of Y (percent of detection) on X (log of concentration) (Snedecor and Cochran, 1967). The intersection of the 50% detection level with the regression line determined the threshold and 95% confidence limits were obtained for each threshold.

Flavor Enhancement by 5'-Nucleotides

Sample Preparation

Nucleotides used throughout this experiment were presented in solution at a concentration of 10 ppm. In addition to the aqueous concentrations of octanal being volatile, the di- and triphosphate nucleotides were relatively unstable in the unbuffered solutions; therefore, initial dilutions were made no more than two hours before presentation to each panelist. Monophosphates are stable under normal conditions (Shimazono, 1964). If a panelist was unable to attend a particular panel session, a makeup panel was held, generally the following day, and fresh samples were prepared.

Each 10 ppm nucleotide solution was prepared by adding 50 mg of 5'-nucleotide to five liters of distilled water. All nucleotides were readily soluble at 10 ppm. Five concentrations of octanal were used. Again, these concentrations were prepared shortly before presentation to the panelists. Each initial dilution was made by dissolving 0.1 g of octanal in 9.9 ml of ethanol. One ml of this solution was added to 9.0 ml of ehtanol and 1 ml of this was added to 999 ml of the nucleotide solution to give a stock solution containing octanal

at 1 ppm, ethanol at 1,000 ppm, and nucleotide at 10 ppm. From this stock solution, 0.05, 0.15, 0.50, 1.5, and 5.0 ml aliquots were added separately to five 500 ml volumetric flasks. The flasks were filled to volume with the nucleotide solution to give the five concentrations of octanal, each in 10 ppm nucleotide solutions.

The first two dilutions in ethanol were required for these reasons: (1) the solubility of octanal in orange juice is greater than in water because of the presence of amphipathic compounds in the juice. Thus, the behavior of octanal with respect to its organoleptic properties in nucleotide solutions containing ethanol should be more representative of its behavior in orange juice; (2) the disodium salts of the nucleotides are far more soluble in water than is octanal. This tends to "salt-out" the octanal creating erroneous vapor pressures and giving false threshold concentrations; (3) it enabled the initial weight of octanal, 0.100 g, to be large enough to achieve three-place accuracy on available balances.

Ethanol was selected as the solvent for octanal based upon the following information: (1) ethanol is a non-toxic solvent; (2) ethanol is a natural constituent of oranges found in extremely high concentrations. Kirchner and Miller (1957) reported that the concentration of ethanol in fresh Valencia juice was 380 ppm; (3) the threshold of ethanol is extremely high. The ten panelists determined its threshold in a 10 ppm solution of ADP to be 215 ppm, over 250,000 times higher than octanal's threshold in ADP.

Kuninaka et al (1964) mentioned the autocatalytic degradation of ADP to IMP. To be certain that ADP had not decomposed to IMP, solutions of ADP prepared in an identical manner as those presented to the panelists were analyzed by differential spectrophotometry, according to a method recommended by Kalckar (1947). The ADP solution had a maximum absorption at 257 nm, which confirmed its presence. No IMP was detected.

Testing Procedure

The ten panelists, six females and four males, used to determine the

influence of selected 5'-nucleotides on the flavor thresholds of octanal were selected from the 75 panelists mentioned above. The ages ranged from 19 - 47 years, with the average being 27.6 years. Two of the male panelists smoked. These panelists were screened and trained as recommended by Kramer at al. (1961) for determining differences.

Penelists were trained by their participation on the previous panels. Selection was based upon the following: (1) their consistency of results between repetitions of the previous panel; (2) their dependability to attend sessions; and, (3) their ability to detect octanal.

Several screening panels were conducted to select the five concentrations to be presented throughout this experiment based upon the following requirements: (1) the log of the concentration had to be equally spaced for analysis purposes; (2) the concentrations had to be centered about the threshold of octanal; (3) the difference between concentrations had to be large enough to allow for the large variation between panelists; and, (4) the difference between the weakest and strongest concentration had to be small enough so that the linear relationship existed. The five concentrations of octanal, 0.1, 0.3, 1.0, 3.0, and 10.0 ppb, were chosen. These concentrations were presented to the panel in ascending order. Each concentration was at least twice as strong as the preceeding one, as recommended by Gregson (1962).

Panel sessions were held once a day between 11:45 A.M. and 12:00 noon, five days per week for seven weeks. Each day a repetition consisting of five concentrations of octanal plus five references was presented to each of the panelists. During the first week, the flavor threshold of octanal in water was established. During each of the following weeks, the flavor thresholds of octanal in one of the six nucleotide solutions (AMP, ADP, ATP, GMP, GDP, and GTP) were determined.

The ten panelists were presented with five pairs of samples which were numbered one through ten. The odd numbered samples were references containing

nucleotide solution only. Concentrations of octanal were presented in ascending order. Panelists were instructed to taste both samples of each pair in numerical order, and indicate if their flavors were alike or different. If they judged that the two samples had different flavors, they were to indicate the number of the sample possessing the stronger flavor. The panelists were not informed of the panel design until the conclusion of the experiment. Also, they were requested not to show or discuss their results with fellow-panelists. This was an attempt to keep the panel design unknown in order to prevent biased answers. Refreshments were served to encourage attendance.

Statistical Analysis

A sample pair received a score of one if a panelist (1) indicated the two samples within the pair as having different flavors, and (2) correctly indicated the sample containing both nucleotide and octanal as having the stronger flavor. If both of these conditions were not met, the sample pair received a score of zero. The scores for each of the five concentrations of octanal were summed for all ten panelists and five repetitions (one repetition each day of the week), giving a total of 50 responses per concentration per nucleotide. This figure was multiplied by two to give the percent of detection. The regression of Y (percent of detection) on X (the log of concentration) was used to determine thresholds. The "t-test" (Snedecor and Cochran, 1967) was used to determine significant difference between the various thresholds.

A 5 x 7 factorial analysis of variance with five blocks (repetitions) was used to analyze for differences in total detection of octanal in each of the six nucleotides plus the control, differences in detection of the five concentrations, and nucleotide x concentration interaction. The responses were summed for each of the panelists per concentration per repetition per nucleotide. Since these sums could only take discreet values from zero through ten, an arcsin transformation was made on all data entered into the analysis to make the values more continuous. Since the arcsin can only be taken for numbers less than or equal to

1.0, the arcsin transformation values were obtained using the following formula where "x" represents the sum of the panelists' results per session:

$$\text{transformation value} = \arcsin(x/10)$$

All statistical analyses related to analyses of variance were performed using the transformation values since they are the numbers of statistical importance, although the actual values have more physical meaning.

A second analysis of variance, 10 x 5 factorial design with seven blocks, was used to analyze difference between the ten panelists, five concentrations, and panelist x concentration interaction. The seven treatments served as blocks. The responses were summed for each repetition per panelist per concentration per treatment. These sums could take values from zero through five; therefore, arcsin transformations were made using the formula:

$$\text{transformation value} = \arcsin(x/5)$$

where "x" represents the sum of each panelist's results for five repetitions of a given concentration of octanal in a given solution. All inverse linear regression and analyses of variance were computed utilizing the IBM 360 computer, located at the University of Florida, Gainesville, Florida. Appendix D lists the statistical formula used to determine all thresholds. Appendices E, F, and G list the Fortran IV programs used to compute inverse linear regression and analyses of variance values.

Interactions of Selected Orange Juice Components

Method and Design

The effects of sugar, acid, pectin, and combinations thereof on flavor thresholds of d-limonene were examined by a small, trained panel. Flavor thresholds were used instead of odor thresholds since the sensation of flavor is more representative of the manner in which orange juice is normally consumed.

The sugars used were sucrose, glucose and fructose (Mallinckrodt

Chemical Works, St. Louis, Mo.) and were all analytical reagent grade. Analytical reagent grade citric acid (Mallinckrodt Chemical Works, St. Louis, Mo.) and practical grade malic acid (Nutritional Biochemical Corporation, Cleveland, Ohio) were also utilized. The pectin, approximately 180 grade, was purchased from Sunkist Growers, Corona, Calif.

The panel consisted of 12 members, 5 males and 7 females, and ranged in age from 22 to 47 years with a mean of 30.6 years. The judges were all students or employees of the Food Science Department at the University of Florida. Judges were chosen from the untrained panel on the basis of their consistency and reliability, thereby making this a screened and trained panel. The same judges were used throughout the study, and they attended every session.

The concentrations of d-limonene tested remained constant throughout the entire study: 0.1, 0.3, 0.5, and 1.0 ppm. These concentrations were the same ones that were presented to the first panel, since they demonstrated an even spread in positive responses.

Preparation and Presentation

The preparation of the sample for this panel was identical to that of the first panel, with the exception of the aqueous medium in which the volatile compounds were dissolved.

The percentage of each particular component utilized was approximately equal to that percentage often found in orange juice: 0.04% pectin (Rouse, 1971), 0.8% acid (Florida Citrus Mutual, 1969) and 9.8% sugar (Florida Citrus Mutual, 1969). The acid solution was composed of 0.1% malic acid and 0.7% citric acid (Ting and Attaway, 1971). The sugar solution contained 4.9% sucrose, 2.35% glucose and 2.55% fructose. (Ting and Attaway, 1971). Solutions containing combinations of these compounds were made using the same concentrations of each component as

were used in the individual solutions.

A different medium was tested each week during an eight week period and included water only; pectin; acid; sugar; pectin and acid; pectin and sugar; sugar and acid; and pectin, sugar and acid. The samples were presented in the same manner as in the first panel. The panel was conducted five days a week from 2:30 to 3:30 p.m. The blanks were placed between samples in order that the blank appeared before each concentration once. The blanks used were identical to the solution used in the reference sample.

The judges were instructed to taste the samples and compare the flavor of each one to the reference. Their judgements were recorded in the same manner as in the first panel. In this case, the answer sheet was placed in an envelope to prevent comparison of the results to those of the other judges.

Statistical Analysis

The statistical analysis for determining threshold values was similar to the one used with the untrained panel. In this analysis, there were four X values (concentrations), which were the same for all the thresholds determined. There were five Y values (% positive responses) corresponding to each of the X values, one for each day of the week. Each Y value consisted of twelve judgments, giving a total of sixty judgments corresponding to each X value. With the aid of a computer, the threshold value, simple correlation coefficient, and confidence limits were calculated for d-limonene in the different solutions, using the method discussed previously.

The threshold values for d-limonene were arranged in a 2^3 factorial design, three components (acid, pectin, and sugar) at two levels (0 and the level present in orange juice). There were three main effects (acid,

pectin, and sugar), three 2-factor interactions (acid x pectin, acid x sugar, and sugar x pectin), and one 3-factor interaction (acid x sugar x pectin). These factors were analyzed by an analysis of variance to determine the effect of each solution on the flavor threshold of d-limonene. If no interactions were present, a t-test would be used to indicate the presence of a significant difference for main effects.

Chi square analysis was used to determine variations present in the panel. Variations for days of the week (repetitions) and judges were tested by comparing the total number of positive responses for each variable. A similar test was used for analyzing variation in correct responses to the blanks. The variables included days of the week (repetitions), judges, positions of the blanks, and the solutions used. Variation of responses to blanks among the eleven compounds in the untrained panel was also examined by a chi square analysis.

Interactions of Selected Volatile Components with Pumpout Orange Juice

Sensory evaluation methods were used to compare the flavor of selected orange oil and essence components added to pumpout orange juice to that of reference (good quality) orange juice.

Acidities of both reference and pumpout orange juices were adjusted, by the addition of citric acid, to obtain Brix:acid ratios close to 15:1. The B/A ratios ranged from 14.8:1 to 15.4:1. Orange oil and essence components were added to pumpout orange juice at concentrations suggested by Kirchner and Miller (1957), Lifshitz et al. (1970), Shaw and Coleman (1973) and Stanley et al. (1961).

Orange juice samples were served to the panelists slightly chilled at temperatures close to 50°F. Three types of panels were used:

1. Trained panel

Eleven judges (6 males and 5 females) ranging in age from 25 to 55 years old evaluated the flavor of juice samples according to two different methods:

A. Three modified pumpout orange juice samples and a reference juice sample were presented to the panelists. Panelists were asked to smell and taste each sample, compare its flavor to that of the reference juice and present their ratings on a scale ranging from 7 for exactly like reference juice to 1 for extremely dissimilar to reference juice. Adams and Birdseye orange juice were used as the good quality reference juices. Reference juice was occasionally introduced as unknown sample to the panelists. Ratings assigned to the reference juice (as unknown sample) were used to adjust pumpout sample ratings on the basis that reference juice should have received the maximum rating of 7. The adjusted ratings were calculated as percents of the maximum rating of 7.

B. Three modified pumpout samples and two reference juices were presented to the panelists. The two reference juices consisted of a pumpout juice assigned a rating of 1 and Birdseye juice assigned a rating of 10. Panelists were asked to smell and taste each sample and assign a rating for its flavor as compared to the two known reference samples. The reference juices were presented occasionally as unknown samples. Ratings were adjusted as mentioned previously.

2. Expert panel

Orange juice samples were presented to expert panels (12 members at the U.S. Department of Agriculture and Subtropical Products Laboratory, Winter Haven, Florida and 14 members at the University

of Florida Research and Education Center at Lake Alfred, Florida). Method of sample presentation was similar to that of method B of the trained panel study. The highest percent maximum ratings were assigned to pumpout juice containing acetaldehyde, citral, ethyl butyrate, d-limonene and octanal (78%); acetaldehyde, citral, ethyl butyrate and d-limonene (75%); and acetaldehyde, citral, ethyl butyrate, d-limonene and α-pinene (70). In addition, the expert panel at Lake Alfred rated the orange juice samples on a hedonic scale. The ratings ranged from 3 to 8 but the average rating scores for the modified pumpout juices were similar to that of good quality reference juice (Birdseye).

3. Untrained panel

One hundred and five untrained panelists ranging in age from 20 to 60 years old and representing different sexes and races were screened for their ability to indicate if the flavor of pumpout juice containing d-limonene or pumpout juice containing d-limonene, acetaldehyde, citral and ethyl butyrate closely resembled the flavor of good quality orange juice (Birdseye). Sixty two panelists were capable of choosing the latter juice over the former. Two samples were of pumpout orange juice containing different combinations of the selected components and a reference juice (Birdseye) were presented to the panelists. Panelists were asked to indicate which modified pumpout juice was closer in flavor to the reference juice. Five sets of panels were conducted using different combinations of selected components.

RESULTS AND DISCUSSION

Flavor and Odor Thresholds

Alcohols, esters and d-limonene

The flavor and odor threshold concentrations for the selected volatile constituents of orange juice which were studied are presented in Tables 10 and 11 respectively. Along with the threshold values are the

Table 10. Flavor thresholds concentrations for selected orange volatiles in distilled water.

Compound	Threshold concentration (ppb)	95% Confidence limits (ppb)	Correlation coefficients
Ethyl butyrate	0.13	0.0008-2.000	.90
Ethyl propionate	4.9	0.9-25	.84
Methyl butyrate	59	23-150	.96
Octyl acetate	210	89-470	.97
Nonyl acetate	270	120-590	.92
Octanol	54	12-230	.94
Decanol	23	9.6-57	.95
Dodecanol	66	25-170	.95
α-Terpineol	320	120-890	.91
Linalool	3.8	1.4-10	.96
d-Limonene	210	140-330	.98
d-carvone	86.0	47.9-151.0	.96
p-cymene	13.3	4.0-40.0	.96
Ethyl vinyl ketone	1.2	0.42-3.0	.94
α-pinene	1013.8	202.3-4971.6	.87

Table 11. Odor threshold concentration for selected orange volatiles in distilled water.

Compound	Threshold concentration (ppb)	95% Confidence limits (ppb)	Correlation coefficients
Ethyl butyrate	.13	.003-4.6	.86
Ethyl propionate	9.9	3.3-29	.97
Methyl butyrate	43	15-120	.95
Octyl acetate	47	14-150	.94
Nonyl acetate	57	19-180	.95
Octanol	190	95-370	.97
Decanol	47	12-180	.93
Dodecanol	73	21-260	.94
α-Terpineol	280	31-2000	.81
Linalool	5.3	1.9-15	.96
d-Limonene	60	20-180	.94
d-carvone	2.7	.001-15454.2	.64
p-cymene	11.4	.006-16360.7	.79
Ethyl vinyl ketone	0.9	.009-80.5	.81
∝- pinene	9.5	.25 -359.3	.90

correlation coefficients and 95% confidence limits. Several of the threshold values for the same compounds reported in the literature are in agreement with the results in Tables 10 and 11. However, there are some that vary greatly, possibly due to methodology. The odor threshold values reported by Buttery et al. (1971) for d-limonene, linalool, and α-terpineol (10, 6 and 350 ppb, respectively) were very similar to those reported here (60, 5.3 and 280 ppb, respectively). Their values were obtained by spraying the compound directly into the nasal cavity. The taste threshold found for ethyl butyrate by Keith and Powers (1968) was 450 ppb, which was approximately 4500 times greater than the value reported in this study, (0.13 ppb). Their method involved starting at a very high concentration and decreasing the concentration until no one could detect the compound. If fatigue from the high concentrations occurred, lower concentraitons were probably not detected, producing a high threshold value. Siek et al. (1969) determined a threshold value for ethyl butyrate (15 ppb) which was 150 times greater than the value reported here. This was possibly due to the methodology they used, where a high concentration was tasted before sampling the other dilutions. Again, fatigue could possibly have produced the high threshold value.

These threshold values can serve as possible indicators of the compounds' flavor significance in orange juice (Patton and Josephson, 1957). If the threshold concentration for a compound is lower than its concentration in the juice, it could have a direct effect on the flavor. The only information available on the concentrations of the compounds used in this study in orange juice is presented in Table 12. Assuming that flavor significance can be indicated in this manner, d-limonene should have a strong influence on the flavor or orange juice. d-Limonene

Table 12. Flavor significance of orange volatiles.

Compound	Threshold (ppb) Flavor	Odor	Concentrations (ppb)[a]
d-limonene	210	60	80,000
α-terpineol	320	280	320
linalool	3.8	5.3	930
n-octanol	54	190	210
n-decanol	23	47	100

[a]Kirchner and Miller (1957).

would then be followed in importance by linalool and decanol. The significance of α-terpineol and octanol might be questioned since their thresholds were near their concentrations. This method of determining flavor significance does not take into consideration interacting effects from other components in the juice. It is possible that the food system could have a masking effect on the compounds which are present in excess of their thresholds, depressing their volatile effect. Those compounds which are present in subthreshold concentrations may be enhanced causing them to contribute to the flavor. Despite these supposedly minor problems this is considered a practical method for assessing flavor (Patton and Josephson, 1957).

The concentrations of alcohols and esters in orange juice are lower than that of aldehydes (Wolford et al., 1971). According to Flath et al. (1971), alcohols in general have the highest thresholds with esters and aldehydes the lowest. If this is the case, then the aldehydes should generally contribute the most to flavor, followed by the esters and then the alcohols.

By observing general trends in a homologous series, more validity may be ascribed to the individual threshold values. A possible correlation exists between odor threshold and the molecular size, at least within series of homologs. Langler and Day (1964) stated that flavor potency increased with corresponding increases in the number of carbons until a maximum was reached, then the flavor potency decreased as the chain length increased. Since the compounds being studied are volatile, their contribution to flavor may be mostly through the sense of smell, making odor thresholds the most likely source of information in relating volatiles to flavor.

Table 13 gives the odor thresholds in relation to chain length for

Table 13. Relationships between numbers of carbon atoms and the odor threshold for related orange volatiles in distilled water.

Compound	Carbon Atom Number	Odor Threshold (ppb)
Methyl butyrate	5	43
Ethyl propionate	5	9.9
Ethyl butyrate	6	.13
Octyl acetate	10	47
Nonyl acetate	11	57
Octanol	8	190
Decanol	10	14
Dodecanol	12	73
Linalool	10	5.3
α-Terpineol	10	280
d-Linomene	10	60

the series of alcohols and esters plus d-limonene. Since there were no significant differences between flavor and odor values, it seems reasonable to consider odor alone. In the group of esters studied, the smaller C_5 molecules (methyl butyrate and ethyl propionate) had higher odor thresholds (43 and 9.9 ppb, respectively) than ethyl butyrate (C_6) which had an odor threshold of .13 ppb. Flath et al. (1967) found similar results with odor thresholds of esters; the smallest maolecule, C_4 (ethyl acetate) had an odor threshold value of 5000 ppb and the C_7 molecule, ethyl 2-methyl butyrate, had a threshold value of .1 ppb. They claimed that several small molecules must be present at one time before the sensation is initiated, where as only one large one need be present to initiate a response. This could account for the need for a higher concentration of small esters to be present for detection. The loss of volatility as molecular weight increased could have accounted for increasing thresholds for the larger molecules.

A similar trend was observed in the alcohols tested, although it was not significant. Octanol (C_8) had a high threshold (190 ppb), followed by a lower threshold (47 ppb) for decanol (C_{10}), and then a higher one again (73 ppb) for dodecanol (C_{12}). Buttery et al. (1971), whose thresholds correlated well with those in this study, also demonstrated this trend. The C_5 and C_6 alcohols had much higher thresholds ranging from 500 to 70 ppb, than the C_{10} alcohols which had thresholds ranging from 3 to 6 ppb. Flath et al. (1967) had high thresholds for C_2 and C_3 alcohols of 100,000 and 9,000 ppb, respectively.

A possible correlation could be found between the number of double bonds and threshold values, utilizing this data and that of Buttery et al. (1969). Four ring structures; d-limonene, α-terpineol, guaiacol and eugenol have 1, 2, 3, and 4 double bonds, respectively. The increase in double bonds appeared to have some degree of correlation with a decrease in odor threshold values, (280, 60, 3 and 6, ppb respectively).

All of these results generally agree with the common theory that odor thresholds are dependent on many parameters, including size, shape, functional groups, and electrophilic and nucleophilic characteristics.

A simple correlation coefficient for the set of points associated with each compound indicated that there was linearity in percentage of positive responses within the range of concentrations tested. The points should fit a straight line in order to give an accurate prediction of the threshold value (Snedecor and Cochran, 1965).

The confidence limits (Tables 10 and 11) are not of equal size on either side of the threshold value due to the conversion of threshold values from their logarithmic forms. The large size of the confidence intervals could be caused by the numerous variables that are present in a sensory testing situation, which are discussed later.

The confidence limits for the flavor and odor thresholds overlap for each compound; therefore, there is no evidence in this study to indicate that odor evaluations produce significantly lower thresholds than flavor evaluations. However, in seven of the eleven comparisons, the odor thresholds were lower, although not significantly. McNamera and Danker (1968) stated that generally much higher concentrations are needed to detect flavor than odor.

Aldehydes

Of the more than 20 isolated and identified aldehydes in fresh orange juice (Table 1), the ten listed in Table 5 were selected for determination of their flavor and odor thresholds. Their selection was based upon the following information: (1) ethanal and butanal are the only two aldehydes to have been detected in relatively large amounts in the aqueous essence phase (Kirchner and Miller, 1957); (2) the most abundant aldehydes in the oil essence phase to have been detected are

octanal, nonanal and decanal (Moshonas and Lund, 1969a); (3) hexanal and trans-2-hexenal have been identified as contributing an immature or "greenish" note to various fruits (Flath et al., 1967; Buttery et al., 1971); (4) citral, as its name implies, has long been associated with the flavor and aroma of citrus; and, (5) perillaldehyde has been reported by Ohta and Hirose (1966) to play an important role in the flavor of Citrus natsudaidai, and Furukawa and Dimizawa (1920) also reported that perillaldehyde readily formed derivatives as much as 2,000 times sweeter than sugar.

The flavor and odor thresholds of the selected aldehydes are listed with their respective confidence limits in Table 14. The values were based upon the data presented in Appendix A and Appendix B. All of these thresholds were in excellent agreement with previously reported thresholds determined in aqueous media (Table 7), with two exceptions. The odor thresholds of decanal and dodecanal determined by Guadagni et al. (1963a) were 0.1 and 2.0 ppm, respectively, while the values in Table 9 are 3.0 and 0.5 ppb, respectively.

The flavor thresholds of octanal and citral were significantly lower than their respective odor thresholds. However, the flavor thresholds of nonanal and trans-2-hexenal were significantly higher than their odor thresholds. Meijboom (1964) had reported that flavor thresholds were lower for 31 aldehydes determined in non-polar media than their odor thresholds. Such was not the case for half of the flavor thresholds determined in a polar (aqueous) media (Table 9). This might be explained by the difference in vapor pressure between aldehydes dissolved in polar media and in non-polar media. As the data in Table 8 indicates, the vapor pressures of aldehydes actually increases as the length of the carbon chain increases when they are dissolved in polar media. The opposite is true for aldehydes dissolved

Table 14. Flavor and odor thresholds of selected aldehydes in aqueous solutions.

Aldehyde	Flavor Thresholds in ppb			Odor Thresholds in ppb		
	Lower Confidence Limit	Probable Threshold	Upper Confidence Limit	Lower Confidence Limit	Probable Threshold	Upper Confidence Limit
Ethanal	19.8	22.0	24.4	13.4	17.0	21.7
Butanal	4.42	5.26	6.27	4.20	15.9	60.2
Hexanal	1.37	3.66	6.78	1.43	9.18	58.9
Octanal	0.50	0.52*	0.54	1.24	1.41*	1.60
Nonanal	3.19	4.25+	5.64	2.12	2.53+	3.02
Decanal	2.67	3.02	3.41	0.79	1.97	4.90
Dodecanal	0.89	1.07	1.29	0.30	0.53	0.95
trans-2-Hexenal	31.0	49.3+	78.4	19.9	24.2+	29.5
Citral	36.3	41.4*	47.3	76.8	85.3*	94.8
Perillaldehyde	16.7	25.3	38.3	19.4	30.1	46.9
Acetaldehyde	-	20.0	-	-	17.0	-
Butyaldehyde	-	-	-	-	10	-
Citronellal	-	35	-	-	66	-
Geranial	-	40	-	-	-	-
Methyl propionate	-	58	-	-	100	-
Mycrene	-	42	-	-	36	-
B-Sinensal	-	11	-	-	3.8	-

* Flavor threshold significantly lower than odor threshold at 95% confidence level

+ Flavor threshold significantly higher than odor threshold at 95% confidence level

(-) Undetermined

in non-polar media (Morrison and Boyd, 1966).

The thresholds reported by Meijboom (1964) were on the order of 10^3 times higher than corresponding thresholds of the same aldehydes determined in aqueous solutions (Tables 7 and 14). In addition to the greater vapor pressure (volatility) of aldehydes in polar solutions, four other factors may also account for the large differences between thresholds when determined in media possessing different polarities:

(1) Most aldehyde molecules are attracted to and held more firmly by non-polar solvents. This makes it less desirable for a molecule to transfer into the aqueous layer surrounding the taste and odor receptor sites, which is necessary before stimulation of the sites can take place (Klopping, 1971). Thus, higher aldehyde concentrations would be required in order to force the molecules into the aqueous phase;

(2) the non-polar medium (oil), itself, undoubtedly blocked many of the receptor sites by its mere presence;

(3) the oil may have had a fatiguing effect on the sites. Zotterman and Diamant (1959) reported that water does not stimulate taste sites and thus, cannot cause fatigue;

(4) perhaps the dissolving of the aldehydes in non-polar media alters their molecular structure to some extent since the shape of the molecule theoretically influences its sensory properties (Amoore et al., 1964).

The seven saturated aldehydes (Table 14) had lower odor and flavor thresholds than the three unsaturated aldehydes. trans-2-Hexenal, which does not possess a distinct primary odor, had an extremely large carry-over effect. This was responsible for the large confidence limit about its threshold, 1.43 to 58.9 ppb (Table 14). Laffort (1963) reported that compounds possessing musk or camphoraceous odors showed above-normal variability (both inter- and intra-panelist variability) in their

threshold determinations.

A definite relationship between threshold value and chain length of the compound cannot be concluded (Meijboom, 1964). However, as the length of the carbon chain increased in the n-alkanal series, the thresholds generally decreased (Table 14). Two exceptions were noted. First, the aldehydes containing carbon atoms in multiples of four, particularly octanal and dodecanal, exhibited lower thresholds than would have been expected by examination of neighboring thresholds. Second, nonanal, the only n-alkanal tested containing an odd number of carbon atoms, had a higher threshold than would have been predicted. Amoore et al. (1964) reported that strong odor seemed to be associated with chains of four and eight carbon atoms of certain aldehydes. Meijboom (1964) observed lower threshold values for even-numbered atoms. He also reported that such alternating effects usually referred to a physical property of crystalline state.

Panelists reported octanal, nonanal, decanal, and dodecanal to possess an orange-like aroma and flavor in 1 ppm aqueous solutions. In addition, a slightly bitter taste was noted. A few of the panelists who smelled perillaldehyde in its pure form mentioned that it had a floral, rose-like aroma, while citral possessed a typical lemon-like aroma. W. H. Perkin, in 1867, classified octanal, nonanal, and decanal as having a floral, orange blossom aroma (Halsey, 1964). He also classified citral as having a fruity aroma. Klopping (1971) classified ethanal as having a pungent odor ar high concentrations and a pleasant, somewhat fruity aroma at low concentrations. Butanal has a putrid odor at most concentrations. Hexanal and trans-2-hexenal contribute the unripe or greenish note to many fruits (Flath et al., 1967: Buttery et al., 1971).

Additional Responses of Panelists

Since panelists were utilized to determine thresholds, fatigue and carry-over effects became two of the more influential factors controlling the degree of precision achieved. Fatigue tended to increase with increased sampling, while carry-over effect increased with decreasing differences between concentrations presented. The number of samples presented and differences between concentrations were restricted by these two factors. However, a paired comparison design in which five concentrations were presented per session enable panelists to consistently discriminate between samples with concentration differences of 10 ppb for all aldehydes presented and with differences of less than 1 ppb for several aldehydes presented. Three aspects of the paired comparison test may have been responsible for the relatively high degree of precision achieved. First, a separate sample was compared to each concentration presented. Second, panelists rinsed their mouths or cleared their noses at least once between concentrations since the references were distilled water. Third, the procedure physically increased the time between sampling of different concentrations simply by requiring the panelists to open two bottles per concentration.

The majority of panelists gave consistent results between repetitions for those concentrations below and above their individual thresholds, and were inconsistent at just those concentrations close to their individual thresholds. However, a few panelists gave very sporadic results between repetitions and did not appear to have narrow individual threshold ranges. Members of this group accounted for a large percentage of the water blanks detected. In other words, they indicated that there was a flavor or odor difference between a pair of samples containing only distilled water. King (1937) commented:

> There was no way of knowing whether or not the judges were trying so hard to

> taste something in the weaker solution
> that their sensations of taste were
> psychological rather than real. pp 211-212

It was noted that several panelists seemed determined to taste or smell a difference between each pair. They often felt that their answers were incorrect if they indicated two samples as tasting or smelling alike when, in fact, there was a difference between them. This seemed to bother most panelists more than indicating two samples as being different when they were alike. While there appeared to be no relationship between age and an individual's ability to detect aldehydes for those between 16 and 50 years of age, all of the panelists over 50 gave very sporadic results and were members of the above-mentioned group. They indicated a large percentage of the water blanks as being different. This observation agreed with Minich et al. (1966), who reported that young panelists were better able to determine differences and gave more consistent results. Gregson (1962) stated that fatigue may have been responsible for panelists' detecting water blanks. Perhaps those panelists over 50 years of age were more subject to fatigue.

Generally, males and females were equally comparable in detecting various aldehydes, except in a few instances, when females detected odors at lower concentrations than did males. For example, when trans-2-hexenal was presented at 40 ppb, 47% (14/30) of the male responses indicated a difference between this concentration and distilled water, while 76% (56/74) of the female responses indicated a difference. Stone et al. (1962) mentioned that some training occured with untrained panelists between repetitions. This was most apparent between initial repetitions of each new aldehyde presented. The panelists seemed to train themselves to detect the odor of each new aldehyde, and so the threshold for most aldehydes declined during the first few repetitions.

Refreshments, orange juice and cookies, were given to each panelist in gratitude for attendance. During the first ten weeks, panel members attended 675 out of a possible 744 times, for a 91% attendance. Guadagni et al. (1963a) mentioned that attendance at their panel sessions was 71%. Thus, the refreshments may have had the effect of increasing attendance.

Flavor Enhancement by 5'-Nucleotides

Octanal was selected as the aldehyde to be studied in connection with flavor enhancement by nucleotides for the following reasons.

(1) Historically, octanal has been the compound described as possessing orange-like flavor and aroma. Perkin, in 1867, described octanal as having an orange blossom aroma (Halsey, 1964).

(2) Octanal has been reported to be in orange juice at higher concentrations than most other aldehydes (Table 6).

(3) Octanal has a low threshold (Tables 7 and 14).

Wagner et al. (1963) reported a drying aftertaste left in the mouth by nucleotides. Panelists reported that the adenosine nucleotides have a chlorinated water aroma and a bitter flavor. They also reported a drying aftertaste. The guanosine nuclectides produced more of a sensation in the mouth rather than actual taste, although some panelists commented on a bitter aftertaste plus the drying aftertaste.

Thresholds of Octanal

Flavor thresholds of octanal were determined in the six 5'-nucleotide solutions and in distilled water (Table 15). These values were determined from the data given in Appendix C. The flavor thresholds of octanal in GMP and AMP were significantly lower that that of the control as determined by t-tests (Snedecor and Cochran, 1967). The flavor enhancing potential of GMP has been well-publicized (Wagner et al.,

1963; Shimazono, 1964; Kuninaka et al., 1964; Luh and Chen, 1969). Thus, it was not unexpected that octanal in 10 ppm solutions of GMP had a significantly lower threshold than the octanal. However, octanal in a 10 ppm solution of ADP had a significantly lower threshold than the control. This threshold, 0.85 ppb, was similar to that established by GMP, which was 0.86 ppb. ADP has been reported by Kuninaka (1960) Shimazono (1964), Kuninaka et al. (1964), Shimazono (1965), and Luh and Chen (1967) to possess little, if any, flavor enhancing or modifying potential.

The flavor enhancing ability of the 5'-nucleotides was influenced by the number of phosphate groups attached, i.e., mono-, di- or tri-phosphates. All of the thresholds determined in the nucleotide solutions were lower than that of the control. The thresholds of octanal in the triphosphate solutions, GTP and ATP, were slightly lower than that of the control. Thus, the triphosphates did little to enhance the flavor of octanal. The diphosphates, on the other hand, had two of the three lowest thresholds. The threshold of octanal in GDP was approximately 30% lower than that of the control, while the threshold of octanal in ADP was approximately 40% lower.

According to Beidler's theory (Beidler, 1966), nucleotides may enhance flavors by unmasking certain flavor receptor sites, allowing them to contribute to stimulus adsorption and taste receptor stimulation. Perhaps the triphosphate molecules are too large and in the process of unmasking the receptor sites, partially conceal them. The diphosphates possess three partially negative charges in their ionized state as opposed to two for the monophosphates. This additional charge may enable the diphosphates to unmask the receptor sites to a greater degree than do the monophosphates.

Kuninaka (1960) and Kuninaka et al. (1964), along with several other

Table 15. Flavor thresholds of octanal determined in aqueous, 10 ppm solutions of selected 5'-nucleotides.

	Concentrations of Octanal in PPB(wt/vol)		
5'-Nucleotide	Lower Confidence Limit[a]	Threshold	Upper Confidence Limit[a]
Control (none)	1.20	1.38	1.59
ATP	0.91	1.22	1.65
GTP	1.00	1.21	1.47
AMP	0.87	1.20	1.64
GDP	0.51	0.99	1.68
GMP	0.57	0.86*	1.29
ADP	0.76	0.85*	0.95

[a]95% confidence limits

*significantly different from control at 95% level as determined by t-test

authors, have stated the following to be requirements for flavor enhancement by nucleotides: (1) the base moiety should be purine; (2) a hydroxy group on the number 6 carbon; (3) the group on the number 2 carbon is optional and has no effect on flavor; and, (4) the 5'-position of ribose is esterified with phosphoric acid. Based upon results of this experiment, an amino group may be present on the number 6 carbon and ribose esterified with a pyrophosphate group, as in the case of ADP.

Woskow (1969) raised the question as to whether nucleotides favorably alter the preference for foods by enhancing the flavor of the food or by the suppression of undesirable "off-flavors." Results of this experiment indicate that the nucleotides ADP, GDP and GMP enhanced the flavor of octanal. However, the results of a panel conducted to determine the flavor threshold of ethanol indicated that ADP masked the flavor of ethanol (Figure 2). Point "A: shows that 35% of the panelists

detected the 10 ppm ethanol in distilled water, while point "B" indicates that just 10% of the panelists detected the same concentration of ethanol in a 10 ppm solution of ADP. This masking effect was probably caused by the bitter flavor of ADP masking the milder ethanol flavor.

Variation in the detection of octanal between repetitions, 5'-nucleotide solutions, concentrations of octanal, and solution x concentration interaction is shown in Table 16. No significant variation (difference) was found between repetitions. Thus, no increase in the ability to detect octanal between days of the week or repetitions was observed with a trained panel. Earlier results had indicated that panelists were most sensitive on Fridays (Mitchell, 1957b). This non-significance is illustrated in more detail by Table 17. Listed are the total number of times octanal was detected by repetition and by day of the week.

The variation between nucleotide solutions was significant (Table 16). Table 18 lists the number of times octanal was detected in each of the six 5'-nucleotide solutions and the control. Duncan's Multiple Range Test revealed that the variation between the number of times octanal was detected in distilled water was significantly lower than the number of times it was detected in the 5'-nucleotide solutions containing GDP, GMP, or ADP. Dunnett's Test, designed to compare a control with all the treatments, also showed a significant variation between the control and ADP, GDP, and GMP. Thus, at identical concentrations, octanal was designated a significantly larger number of times as having a stronger flavor when sampled in solutions containing GMP, GDP, or ADP, than when sampled in distilled water.

Variation between concentrations was significant (Table 16). The Duncan Multiple Range Test indicated the number of detections by the panel of each concentration was significantly higher than the preceeding

Table 16. Analysis of variation in the detection of octanal between repetitions, nucleotide solutions, and octanal concentrations.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F - test
Total	174	26.4710		
Repetitions(Blocks)	4	0.1350	0.0338	1.73
Treatments	34	23.6818	0.6965	35.69*
Nucleotides	6	0.3463	0.0577	2.96*
Concentrations	4	22.9657	5.7414	294.19*
Linear	1	22.7357	22.7357	1164.99*
Quadratic	1	0.0265	0.0265	1.36
Cubic	1	0.1630	0.1630	8.35*
Quartic	1	0.0399	0.0399	2.04
Sol. x Conc.	24	0.3699	0.0154	0.79
Error	136	2.6541	0.0195	

*significance at 95% level

concentration (Table 19). The majority of the variation between concentrations was due to a linear relationship between percent of detection and concentration as opposed to quadratic, cubic, etc. The sum of squares due to the different concentrations presented was 22.97. Of this, 22.47 (over 99%) was due to a linear relationship between concentrations and percent detected. The results shown in Figure 3 are an example of the linear relationship observed throughout this experiment. Such linear response indicated that little, if any, carry-over effects existed. Although the variance due to cubic effect was significant, it accounted for less than 1% of the concentration's sum of squares and was undoubtedly caused by experimental design and a small error sum of squares (Table 16). This relatively small error value possibly supports Kramer's recommendation (Kramer et al., 1961) that a small screened and trained

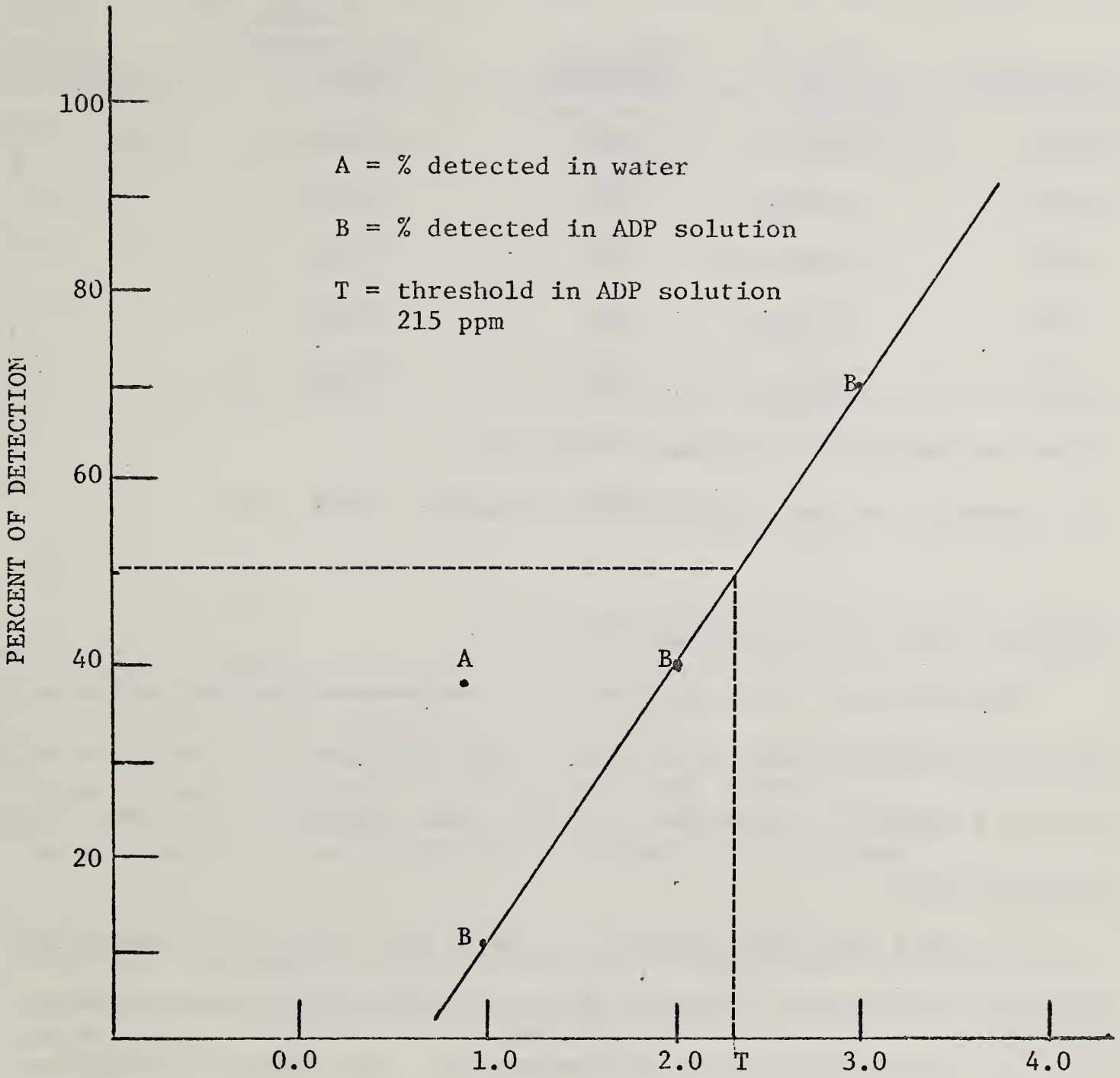


Figure 2. Detection of ethanol in a 10 ppm solution of ADP and in distilled water.

Table 17. Variation in the detection of octanal between repetitions.

Repetition	Day of Week	Number of Detections	Arcsin Transformation Values	Duncan Multiple Range Test[a]
First	Monday	181	20.0	
Second	Tuesday	162	18.3	
Third	Wednesday	162	18.6	*
Fourth	Thursday	167	18.8	
Fifth	Friday	172	19.5	

[a]based on analysis of variance in Table 16

*non-connected values significantly different at 95% level

panel was best for determining differences.

No significant interaction was observed between the nucleotide solutions and concentrations of octanal. This indicated that panelists scored identical nucleotide solutions similarly when sampled in the same octanal concentrations.

A trained panel consisting of 10 judges was utilized to examine the influence of ethanol, monosodium glutamate (MSG) or potassium chloride (KCl) on flavor threshold of aqueous octanal. The flavor threshold was evident as octanal was used in concentrations of 3.0 or 10.0 ppb (Table 20). The use of any of these additives did not seem to increase the percent detection of octanal. In most cases, lower detection percentages were found upon the use of these additives to 10 ppb of octanal. Octanal is present in orange juice at a concentration of 60 ppb. There is the possibility that beneficial effects may be obtained if these additives were added to a higher concentration of octanal solution.

Additional Responses of Panelists

A second analysis of variance (Table 21) demonstrates the variation

Table 18. Variation in the detection of octanal between nucleotide solutions and control.

5'-Nucleotide in Solution at 10ppm	Number of Detections	Arcsin Transformation Values	Duncan Multiple Range Test[a]	Dunnett's Test[a]
Control	111	12.2	-	
ATP	115	12.9	-	NS
GTP	115	13.0	*	NS
AMP	116	13.1	- *	NS
GDP	125	14.6	-	+
GMP	131	15.0	-	+
ADP	131	15.4	-	+

[a] based on analysis of variance in Table 16

NS not significantly different fron control

\+ significantly different from control at 95% level

* non-connected values significantly different at 95% level

Table 19. Variation in the detection of octanal between concentrations.

Concentration (ppb)	Number of Detections	Arcsin Transformation Values[a]
0.10	28	5.71 *
0.30	78	16.7 *
1.00	184	47.5 *
3.00	254	72.0 *
10.0	300	89.2 *

[a] based on analysis of variance in Table 16

* non-connected values significantly different at 95% level

Table 20. The effect of additives on percent detection of octanal present in distilled water.

Additive	Concentration of Octanal (ppb)				
	0.1	0.3	1.0	3.0	10.0
Octanal only	10	4	32	48	80
Oct. + Alcohol	8	12	36	54	84
Oct. + MSG	14	22	40	72	72
Oct. + KCl	10	4	30	54	56

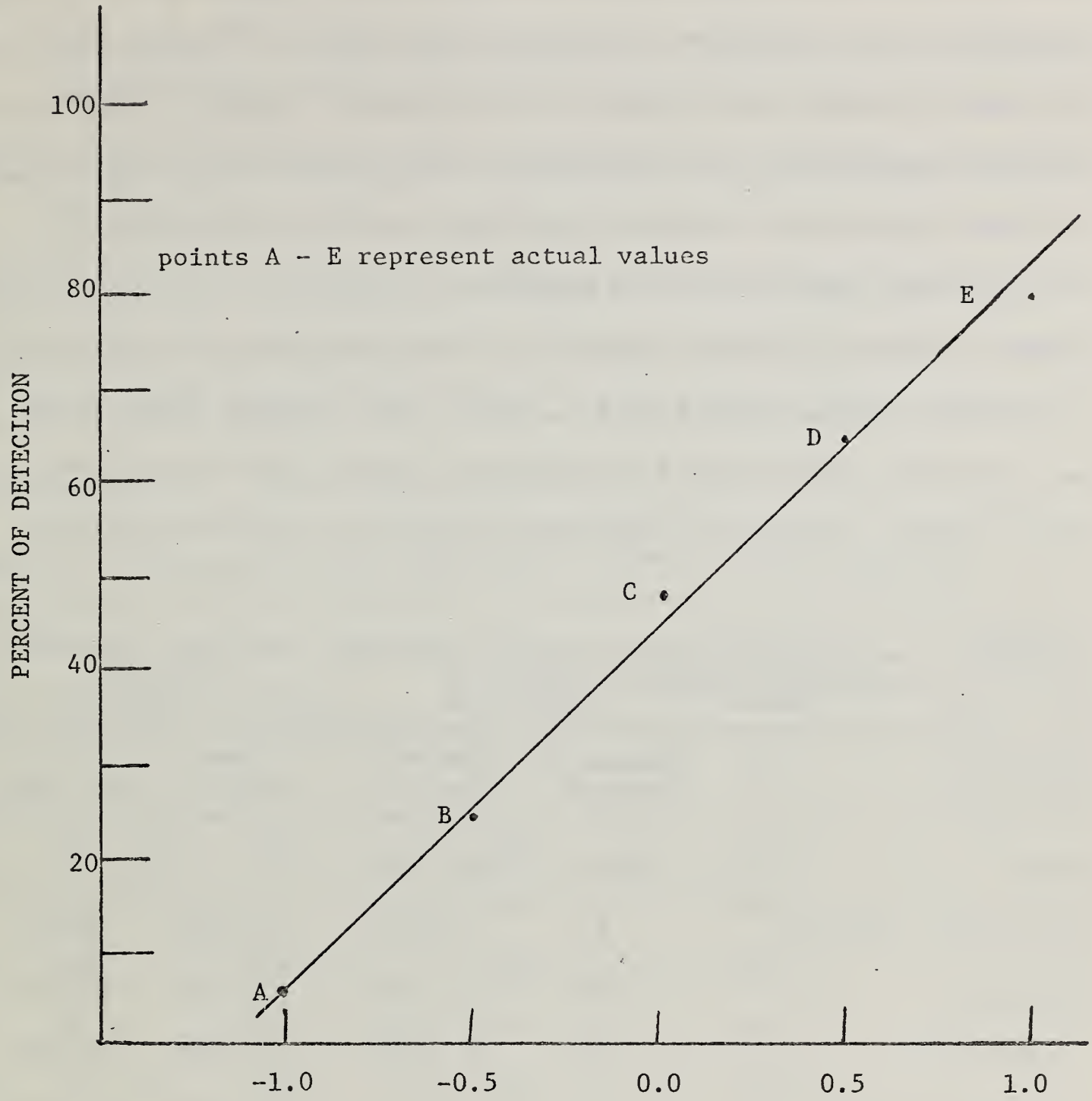


Figure 3. Linear relationship between percent of detection versus log of concentration of octanal in ppb.

between panelists. Lea and Swoboda (1955) and Laffort (1964) commented on the considerable variation between individual panel members. The magnitude of this variation is further illustrated in Table 22, where the number of times octanal was correctly detected by the individual panelists ranged from 11 to 127 samples with five levels of significance. Panelists who detected octanal the greatest number of times were all female, while three of the four panelists who detected octanal the least number of times were males (Table 22). This result was just the opposite of Minich's results (Minich et al., 1966). His findings showed young male panelists (19-30 years) better able to detect differences than young females. Perhaps the difference between the two sexes in their

Table 21. Analysis of variance between nucleotide solutions, panelists, and octanal concentrations.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F - Test
Total	349	141.5181		
Nucleotides (Blocks)	6	0.9833	0.1639	2.83*
Treatments	49	123.5086	2.5206	43.53*
Panelists	9	31.6655	3.5184	60.76*
Concentrations	4	71.8284	17.9571	310.09*
Panel. x Concen.	36	20.0157	0.5560	9.60*
Error	294	17.0252	0.0579	

*significant at 95% level

ability to detect flavors and aromas is not so great as the differences between individuals within the sexes. Panelist number 8, a female, detected octanal correctly just 64 times. However, she was the only one to indicate any sample containing nucleotide as having a stronger flavor than the sample containing nucleotide plus octanal rather consistently.

Several authors (Woskow, 1969) have suggested that nucleotides may suppress flavor rather than enhance it. This may have been the case for this panelist. She was very sensitive to the flavor of nucleotides, to the point, perhaps, of being overly sensitive. She commented several times on the extreme bitterness of the nucleotide solution, particularly the guanosine nucleotides, when the other panelists were barely able to detect a flavor. The addition of octanal to the nucleotide solution may have masked the bitterness of the solution for this panelist. Thus, the sample with octanal appeared to have less flavor than the sample without octanal. She was the only panelist of the ten able to detect

Table 22. Variation in the detection of octanal between panelists.

Panelist				Number of Detections	Arcsin Transformation Values	Duncan Multiple Range Test[b]
No.	Sex	Age[a]	Smokes			
1	F	2	no	127	36.2	
2	F	3	no	119	34.3	
3	F	4	no	108	32.3	
4	F	1	no	112	29.1	
5	M	1	no	107	28.8	
6	F	2	no	80	22.8	
7	M	4	no	75	17.7	
8	F	1	no	64	17.7	
9	M	2	yes	41	10.2	
10	M	2	yes	11	2.2	

[a]ages were divided into four groups: 1=23 years; 2=23-27 years; 3=27-31 years; 4=31 years.

[b]based upon analysis of variance in Table 21

*non-connected values significantly different at 95% level

ethanol at a concentration of 10 ppm in the ADP solution (Figure 2) and was responsible for the 10% detected at 10 ppm.

In order to determine if the nucleotide enhanced or masked the flavor of octanal, panelists were required to specify which sample in each pair possessed the stronger or more intense flavor. A response labelling the reference sample as having the stronger flavor indicated a masking effect in the aldehyde-nucleotide solution, since this solution possessed less flavor than did the nucleotide reference. A response labelling the aldehyde-nucleotide solution as having the stronger flavor indicated an additive or enhancing effect between the nucleotide and octanal. Without the identification of the sample possessing the stronger flavor, some results would have been interpreted much differently.

Age did not affect the panelists' ability to detect octanal in the nucleotide solutions (Table 22). This may be due to the fact that the panelists were relatively young. Just two members were over 31 years of age and all were younger than 50 years. The two panel mambers who smoked (cigars) detected octanal the fewest number of times. Panelist number 10 smoked more regularly than did panelist number 9. It is possible that smoking fatigues the organoleptic receptors temporarily or permanently, but a more in-depth panel study would be required to either prove or disprove this hypothesis.

The second analysis of variance (Table 21) shows singificant interaction between panelists and concentrations. Kramer et al. (1967) mentioned that panelist x concentration interaction, when found significant, indicates that individual panelists score the same concentrations differently. This was true. Due to the large variation between panelists, some consistently detected octanal at lower concentrations than

did other panelists.

Interactions of Orange Juice Components

A highly trained panel was used to evaluate the effect of selected non-volatile components present in orange juice on d-limonene, the most abundant volatile in orange juice. Table 23 contains the flavor threshold values for d-limonene in various solutions of acid, pectin, and sugar. The simple correlation coefficients are included to show linearity, and the confidence limits to estimate variation. The threshold for d-limonene in water is very close to the threshold established for d-limonene by the untrained panel. These values were established almost one year apart, indicating good reproducibility. The methods were identical except that the composition of the panel varied.

Flavor thresholds of d-limonene in a 2^3 factorial design for analysis of the effects from different solutions are presented in Table 24. The individual components did not significantly vary in their effect on the threshold of d-limonene when in combinations with other components according to an analysis of variance. Since each component behaved similarly in different solutions the average effect of each component was evaluated using a t-test (Table 25). The average of the threshold values calculated in solutions containing acid was significantly higher than the average of the threshold values determined in solutions not containing acid. The t-test indicated no significant changes in the average threshold values when sugar or pectin was added. However, the results in Table 25 demonstrated a tendency for the threshold values to be lower in those solutions containing pectin than those without. The opposite tendency was shown for sugar, even though it was not significant. The threshold values, on the average, were higher in solutions containing sugar than in those lacking sugar.

Table 23. Flavor thresholds for d-limonene in aqueous solutions containing pectin, acid, sugar and combinations thereof.

Solutions	Threshold concentration (ppm)	95% Confidence limits (ppm)	Correlation coefficients
Water only	.21	0.05-0.79	.82
Pectin	.22	0.07-0.61	.88
Acid	.41	0.17-0.99	.91
Sugar	.35	0.12-0.96	.89
Pectin & acid	.31	0.08-1.20	.83
Pectin & sugar	.23	0.08-0.64	.89
Acid & sugar	.38	0.14-1.10	.88
Pectin, acid & sugar	.36	0.13-1.00	.89

Table 24. Effect of pectin, acid and sugar on the flavor threshold of d-limonene (ppm).

	Pectin 0%		Pectin 0.04%	
	sugar 0%	sugar 9.8%	sugar 0%	sugar 9.8%
acid 0%	.21	.35	.22	.23
acid 0.8%	.41	.38	.31	.36

Table 25. Average flavor threshold of d-limonene in aqueous solutions with and without pectin, acid and sugar.

Solution	Average Thresholds (ppm)
With Acid	0.36
Without acid	0.25*
With Sugar	0.33
Without Sugar	0.28
With Pectin	0.28
Without Pectin	0.34

* significantly different at the 95% level.

In order to make a definite statement on the effect of non-volatile components in orange juice on the volatile components, many more volatiles must be studied. Since d-limonene is by no means representative of all of the volatile components, it can only be hypothesized that these results indicate that acid may play an important role in masking the characteristic flavor components (volatiles) in orange juice. This is in agreement with the findings of Pangborn (1960) who found that in fruit nectar the greater the acidity, the greater the depressing effect on the intensity of the compound added. Bennett et al. (1965) found the same effect of acid on glycerol.

The fact that sugar had a slight tendency to raise the threshold of d-limonene (although not significantly) agreed with other similar studies. Valdes et al. (1956) claimed that sweetness beyond that imparted by 15.0% sugar interfered with flavor perception. Hinreiner et al. (1955a) claimed that sucrose increased the threshold for alochol.

The pectin did not actually decrease the threshold of d-limonene, but it appeared to depress the effect of acid and sugar on d-limonene. Bennett et al. (1965) stated that the fat in cream could have depressed the apparent acid taste and allowed diacetyl to be perceived more easily. Since pectin is generally regarded as a thickening agent, its effect on the threshold may be due to textural properties. If in fact the texture of pectin does play an important role, then perhaps an increase in consistency of the solutions may depress the effect sugar and acid have on the volatile components.

Variables Present in the Panel

The two panels were not run with the sole purpose of discerning information pertaining to sensory panels. However, some generalizations from the results pertaining to the variables encountered in running a sensory panel can be made.

The design of the trained panel was more controlled than the untrained panel. Therefore, most of the information pertaining to panel variables was obtained from the highly trained panel. The variables were evaluated using two sources of information: the number of positive responses to the concentrations, and the number of correct responses to the blanks. The number of positive responses could reflect the ability to detect low concentrations. The number of correct responses to the blanks could be a possible measure of the accuracy which is present in panelist sampling. Therefore, the response to the blanks will be referred to throughout the rest of the report as a degree of accuracy. Responses to blanks have not been previously used to examine variations in panel results.

The results of a chi square analysis to determine significant variation in panel responses are presented in Table 26. These results are illustrated in Figures 4 through 6. Table 26 indicates that there is a highly significant variation among the panelists' ability to detect concentrations. This is in agreement with the fact that individuals vary in their ability to detect small differences in concentrations (McNamara and Danker, 1968). However, the variation among the panelists on the number of correct responses they made to the blanks was only significant at the 95% level (Table 26). This would appear to indicate that panelists do not vary as much in accuracy as they do in the ability to detect low concentrations. The response variations are illustrated in Figure 4.

Table 26 also indicates a lack of variation in positive responses to concentrations among the days of the week. This is perhaps an indication that judges do not generally vary in their abilities to detect minimum concentrations during the week. However, Figure 5

Table 26. Chi square analysis of variation of flavor panels in detecting concentrations and correct responses to water blanks.

Source	Concentrations	Blanks
Panelists	34.19**	19.53*
Days	5.14	4.58
Positions	-	17.53**
Solutions	-	34.18**

* significant difference at .05 level

** significant difference at .01 level

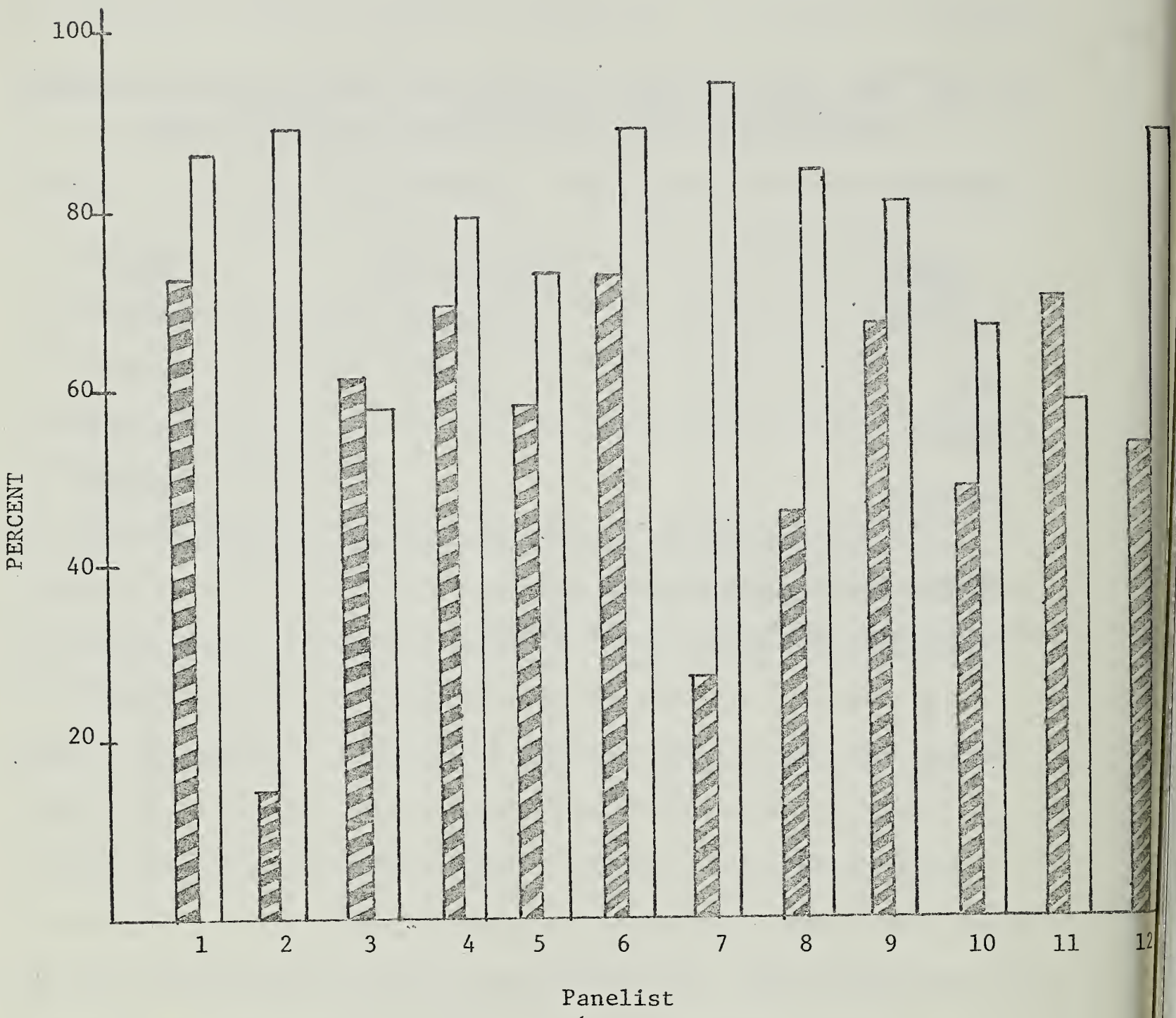


Figure 4. Variation within individual judges in percentage of correct response to blanks and positive response to concentrations.

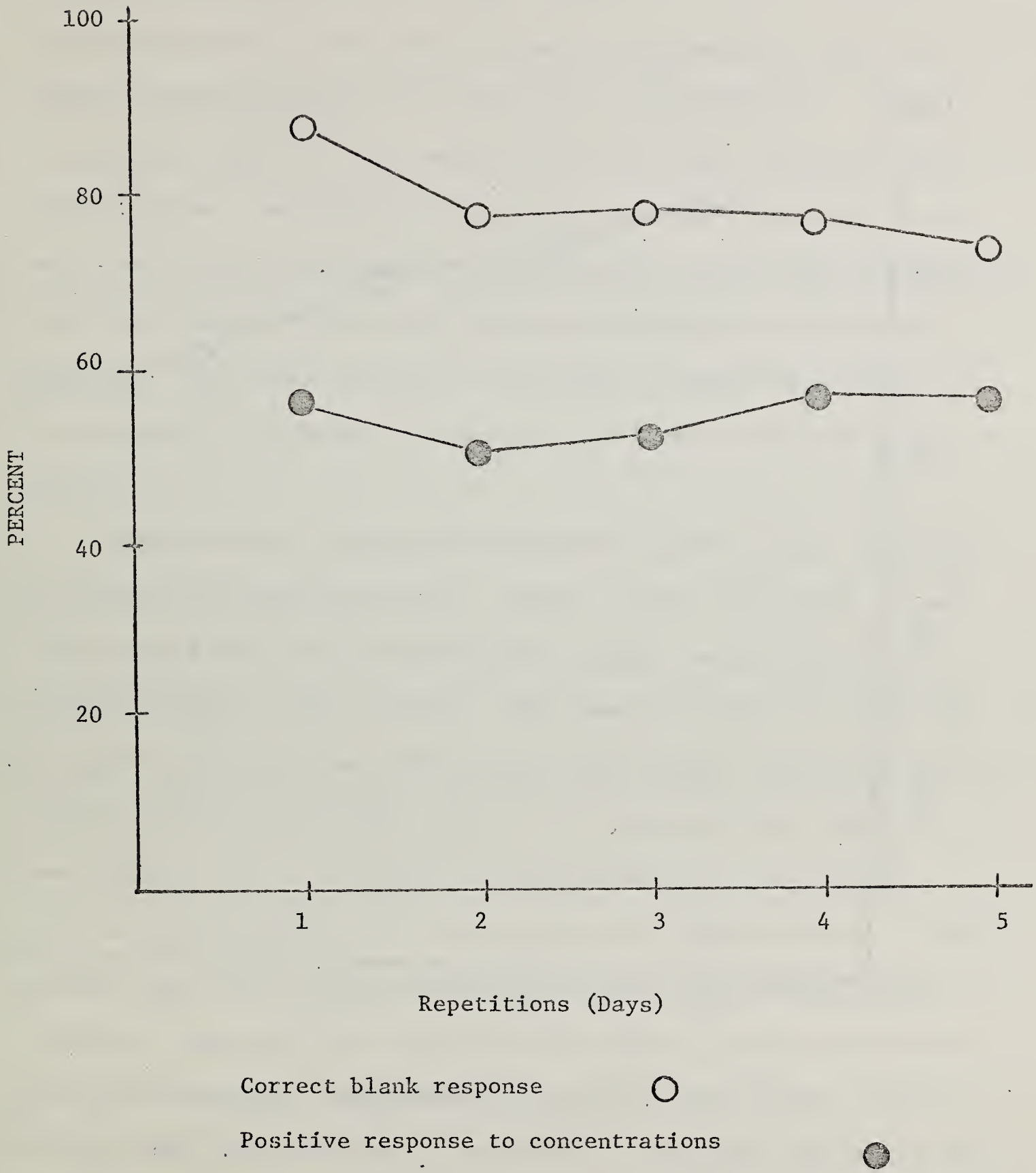


Figure 5. Variations among repetitions (week days) for percentage of correct blank response and positive response to concentrations.

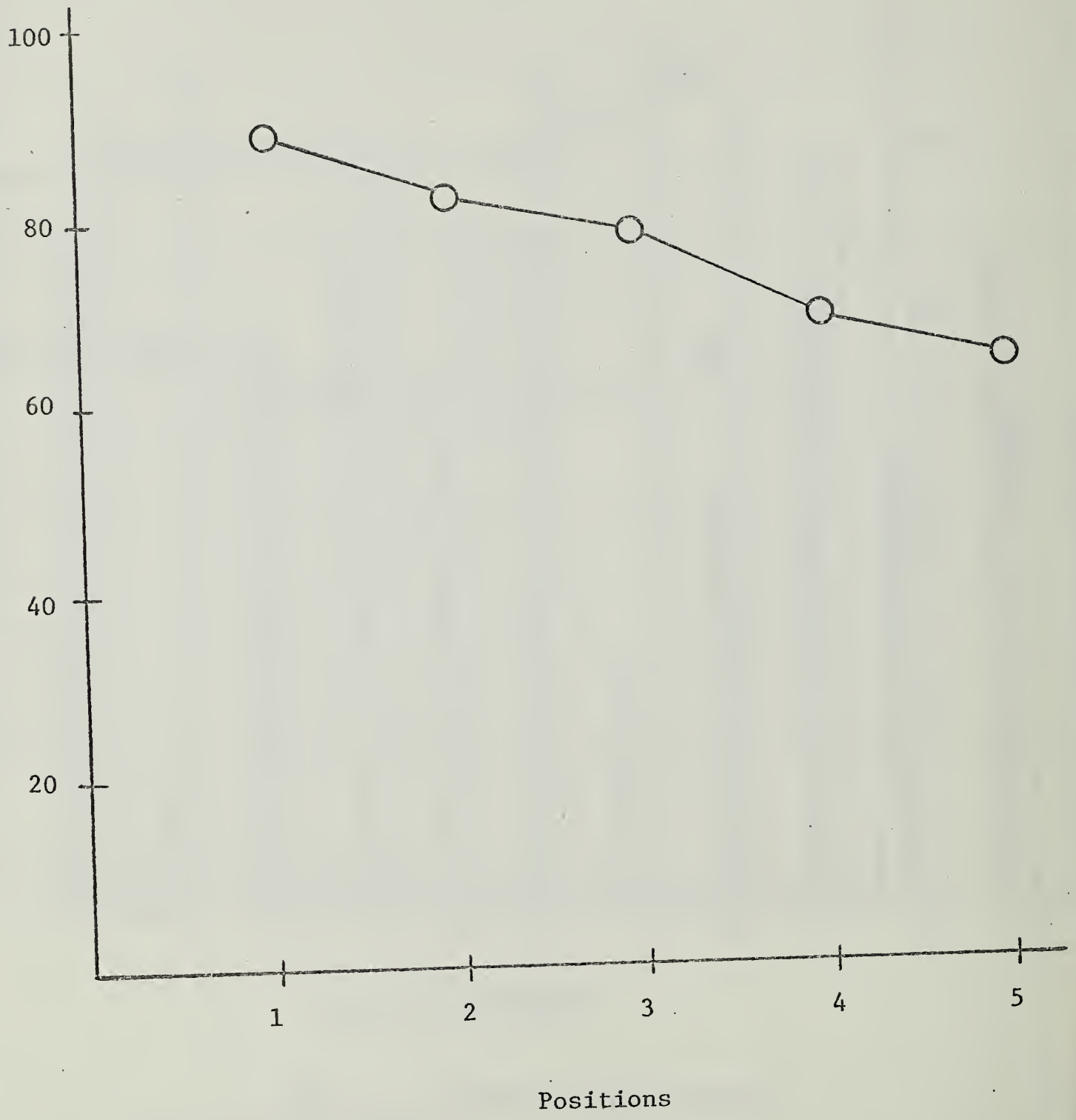


Figure 6. Variations of correct blank responses among sample positions after the reference.

shows a tendency, although not significant, for a high number of positive responses on Monday, followed by a decline on Tuesday, with a gradual increase toward the end of the week. Similar results were obtained by Schinneller (1972). The number of correct responses to blanks also showed no significant variation from day to day (Table 26). Again, it may be an indication that accuracy does not vary during the week. Figure 5 shows a decrease in correct responses toward the end of the week. Both of the lines on the graph probably result from psychological variations throughout the week involving motivation in maintaining interest. The slight increase in the number of positive responses is accompanied by a decrease in accuracy, resulting in essentially no effect.

Table 26 shows a significant difference in the correct responses to blanks in different positions. Figure 6 would then appear to mean that as the blanks are placed after the higher concentrations, responses to them are less accurate. This loss in accuracy can possibly be caused by a carry-over effect which could leave a taste from the previous sample in the mouth, making the following blank appear to have a flavor also. A panelist's anticipation of a flavor in the last few concentrations could also account for this phenomenon. Judges realized that the concentrations were arranged in increasing order; therefore, they could have expected to be able to detect a difference in the last samples, but not necessarily in the first ones. If this was the case, the percentage of responses to the latter samples could have reflected high results.

A significant variation in correct responses to blanks was found among the different solutions in which the threshold of d-limonene was determined. This could have indicated that the different solutions affected the accuracy with which a judge could detect small differences.

According to Table 27, accuracy appeared to be high in solutions of water alone and in the aqueous solution of sugar alone. The results of the sugar solution were rather surprising. Perhaps sugar caused the d-limonene to have a more definite flavor which could not be easily mistaken. The solutions containing acid showed a definite decrease in the accuracy of response. The acid taste was probably strong enough to cause a certain amount of confusion, thus decreasing the panelist's abilities to detect small differences. A similar observation was made by Bennett et al. (1965), who found that acetic acid has such a strong effect on the threshold of diacetyl in sour cream that the diacetyl threshold could not be accurately established in sour cream.

A chi square analysis was also utilized to analyze the results of the large untrained panel for variation among correct responses to the water blanks among the eleven different compounds. A chi square value of 500.5 was found, which indicated a very highly significant difference among the different compounds. The percentage ranged from 71.6 to 97.6 correct responses for flavor, and 78.0 to 97.5 for odor. This probably indicated accuracy in detecting blanks could have depended on the volatile compound being tested.

The untrained panel was also analyzed by chi square tests for differences in blank responses between flavor and odor. The chi square value was 0.40, which indicated no difference in percentages of correct responses to blanks. This supported the previous conclusion that there was no significant difference between flavor and odor thresholds.

There is a possibility that other variations which are not measurable statistically were present, including slight changes in room temperature (75-80°F) and occasional presence of foreign odors

Table 27. Percentage of correct responses to blanks in different solutions.

Solution	Percentages
Water	90.0
Pectin	72.9
Acid	66.7
Sugar	94.4
Pectin x acid	68.7
Acid x acid	76.7
Acid x sugar	73.3
Pectin x acid x sugar	80.0

in the panel room. These variables could not be controlled in this testing situation.

Flavor Components and Orange Juice

1. A trained panel of 10 people was utilized to compare the flavor of selected compounds to that of a reference orange juice. Each compound was presented to the panelists at 3 different concentrations: threshold level, 10 times the threshold level and that equivalent to the amount normally present in good quality orange juice. A water blank with no compound added to it was used in each case. Concentrations equivalent to those found in orange juice exceeded the threshold values for most compounds with the exception of citral, dodecanal, nonanal and B-sinsesal. Each panelist was requested to rate each concentration of each compound according to a scale ranging from 1 for no similarity to reference juice to 7 for exactly like reference juice. A sample of such a questionnaire is shown in Fig. 7. Results shown in Table 28 indicate that: a) highest ratings were always associated with the largest concentration used, b) Ethyl buterate, d-limonene, linalool or octanal used at concentrations equivalent to those present in orange juice received the highest ratings, and c) all the ratings ranged from moderately not like reference juice (2) to neither like or dislike reference juice (4).

2. A trained panel of 10 persons was utilized to compare the flavor of selected compounds in water and in pump out orange juice to that of a reference orange juice. Reference juice was served to the panelists 30 to 60 minutes prior to flavor comparisons. A water blank or plain pump out juice with no compounds added was used in each case. Compounds were added at the concentrations shown in Table 38. Each panel member

Table 28. Flavor evaluation[1] of selected compounds in comparison with orange juice

Compound	Amount			
	None	Threshold	Threshold x10	Equivalent to O.J. Concent.
Acetaldehyde	1.3 b	1.6 b	2.8 a	2.4 a
Citral	1.2 b	1.6 b	2.3 a	1.0 b
Decanal	1.5 b	1.3 b	2.0 a	2.5 a
Dodecanal	1.4 a	1.7 a	2.4 a	2.4 a
Ethyl butyrate	1.4 b	1.4 b	2.0 b	3.0 a
d-limonene	1.4 c	1.5 c	2.3 b	3.3 a
Linalool	1.1 c	1.3 c	3.0 a	3.3 a
Nonanal	1.5 b	1.1 b	2.0 a	1.4 b
Octanal	1.3 b	1.3 b	2.4 ab	2.8 a
B-Sinensal	1.2 c	1.8 b	2.9 a	1.3 c

[1] Rating scale 1 = no similarity to orange juice
7 = exactly like reference juice

Means, within each row, followed by the same letter are not statistically different at the 95% confidence level.

Figure 7.

Name_____

Compare each sample with the reference sample previously presented, on the basis of orange juice flavor, disregarding color and texture. Indicate where you feel the flavor of each of the samples lies from "exactly like orange juice" to "no similarity to orange juice".

		Sample Number			
FLAVOR		1	2	3	4
Exactly like reference juice	7				
	6				
	5				
	4				
	3				
	2				
No similarity to reference juice	1				

was requested to rate each sample on a scale ranging from 1 for no similarity to reference juice to 7 for exactly like reference juice. It was assumed that the panelists had acquired a definite mental image of the reference juice at times of sample presentations. The results of flavor comparisons are shown in Table 29. Several general statements could be made about these results:

1. Sensory ratings of the compounds added to pump out orange juice were higher than those present in water.
2. Any compound added to water received higher rating than that given to pure water. This was not necessarily true as the compounds were added to pump out juice. Some compounds or their combinations improved the flavor of pump out juice, others did not change its degree of acceptability and few

resulted in lower ratings of the modified pump out juice.

3. Acetaldehyde, ethyl butyrate, citral and their combinations with other compounds resulted in improving the flavor of the pump out juice, Linalool, d-limonene, ethyl vinyl ketone, decanal or their combinations lowered the degree of acceptability of the base juice.

4. The variations in the degree of acceptability of the pump out juice at different times of presentation to the panelists could be due to the variations in degree Brix, acidity and their ratios among different batches of the pump out. These variations were also found for the reference juice. On several occasions the panelists described the pump out juice as sweeter than the reference juice. The rigid control of the degrees Brix and acidity of both types of juices should be attempted prior to presentation to the panelists.

3. A trained panel of 11 judges was utilized to compare the flavor of selected compounds in pump out orange juice to that of reference orange juice. Three pump out juice samples were served to each panelist along with the reference juice. Plain pump out juice with no compounds added to it and reference juice were introduced occasionally as unknown samples to the panelists. Acidity of both the pump out and reference juices were adjusted, by the addition of citric acid, to obtain Brix:acid ratio close to 15:1. The acidities and degrees Brix for both types of juice before and after adjustment are shown in Table 30. Compounds were added, at concentrations shown in Table 31, to pump out juice.

Panelists were requested to smell and taste each sample and present their ratings on a scale ranging from 7 for exactly like reference juice to 1 extremely dissimilar to reference juice. The results of the panel

Table 29. Flavor evaluation[1] of pure compounds in distilled water and pump out juice.

Compound	Water		Pump Out	
	Control	Sample	Control	Sample
Citral	---	---	4.4	4.8
Acetaldehyde	---	---	4.6	4.9
Ethyl butyrate	---	---	4.4	4.7
Octanal	---	---	4.6	4.8
d-limonene	---	---	4.6	3.2
Linalool	---	---	4.4	4.1
Ethyl vinyl ketone	---	---	3.5	1.4
Dodecanal	---	---	3.5	3.3
Decanal	---	---	3.5	2.4
Nonenal	---	---	3.5	3.7
∝-pinene	---	---	3.5	3.8
Ethyl butyrate, linalool	1.3	2.1	4.1	4.1
Octanal, linalool	1.3	2.0	4.1	3.7
Linonene, octanal	1.3	2.6	4.1	3.3
Acetaldehyde, octanal	1.3	2.0	4.0	4.2
Acetaldehyde, linalool	1.3	2.3	4.0	4.1
Acetaldehyde, limonene	1.3	2.4	4.0	3.8
Acetaldehyde, ethyl butyrate	1.3	2.6	4.0	4.4
Limonene, linalool, octanal	1.3	2.7	4.1	3.7
Ethyl butyrate, linalool, octanal	1.1	2.2	4.5	4.6
Limonene, linalool, acetaldehyde	1.2	2.5	3.7	3.9
Limonene, ehtyl butyrate, acetaldehyde	1.2	3.1	3.7	4.1
Limonene, octanal, acetaldehyde	1.2	2.2	3.7	3.7
Ethyl butyrate, octanal, acetaldehyde	1.3	2.2	4.3	4.5
Linalool, octanal, acetaldehyde	1.3	2.2	4.3	4.2
Ethyl butyrate, linalool, acetaldehyde	1.1	2.0	4.5	4.9
Limonene, linalool, ethyl butyrate, acetaldehyde	1.1	2.4	4.1	4.5
Limonene, linalool, octanal acetaldehyde	1.3	2.3	4.3	3.5
Limonene, ethyl butyrate, octanal, acetaldehyde	1.3	2.7	4.3	4.7
Linalool, ethyl butyrate, octanal, acetaldehyde	1.1	2.2	4.5	4.8
Limonene, linalool, ehtyl butyrate, octanal, acetaldehyde	1.2	3.0	3.7	4.2

[1] Rating scale 1 = no similarity to reference orange juice.
7 = similar to reference orange juice.

Table 30. Degrees Brix, percent acidity and Brix:acid ratios of reference and pumpout orange juice.

	Reference Orange Juice					Pumpout				
		ACIDITY		B/A RATIO			ACIDITY		B/A RATIO	
Date	Brix	Before Adjustment	After Adjustment	Before Adjustment	After Adjustment	Brix	Before Adjustment	After Adjustment	Before Adjustment	After Adjustment
9-18-72	11.95	.725	.822	17.20	14.50	12.20	.750	.834	17.00	15.20
9-21-72	12.47	.765	.847	16.30	14.70	13.32	.815	.865	16.30	15.30
9-25-72	12.50	.727	.831	17.21	15.06	15.32	.916	1.016	16.70	15.10
9-27-72	11.93	.709	.796	16.81	14.98	13.43	.809	.878	16.60	15.10
9-28-72	12.23	.709	.809	17.37	15.10	12.23	.740	.834	16.60	14.70
10- 4-72	12.16	.700	.827	17.37	14.70	11.96	.721	.809	16.60	14.80
10- 9-72	11.66	.690	.794	16.90	14.70	12.25	.737	.816	16.60	15.00
10-19-72	11.81	.720	.787	16.40	15.00	12.25	.723	.840	16.90	14.60
10-24-72	12.66	.755	.845	16.76	15.10	12.08	.733	.819	16.50	14.70
10-30-72	12.38	.736	.822	16.82	15.10	12.31	.760	.760	14.90	14.90
11- 6-72	10.96	.645	.726	16.98	15.09	12.26	.734	.828	16.70	14.80
11- 8-72	12.56	.742	.838	16.93	14.98	12.06	.779	.832	15.50	14.50
11-15-72	12.36	.793	.810	15.60	15.26					
11-21-72	12.53	.790	.819	15.86	15.30					
11-28-72	12.41	.800	.832	15.50	14.90	12.31	.777	.865	15.80	14.30
11-29-72	12.41	.777	.829	15.98	14.97	12.21	.770	.806	15.80	15.10
12- 6-72	12.01	.747	.825	16.08	14.56	12.88	.805	.849	16.00	15.20
12-11-72	12.61	.780	.842	16.17	14.98					

Table 31. Concentrations (ppb) of selected compounds in pumpout juice.

Compound	ppb in juice	Reference
Acetaldehyde	3000	Kirchner, Miller 1957
Citral	780	Shaw
Citronellal	140	Shaw
Decanal	720	Shaw
Dodecanal	120	Shaw
Ethyl butyrate	400	
d-limonene	190,200	Shaw
Linalool	840	Shaw
Myrcene	5,300	Shaw
Nonanal	10	Stanley et al. 1961
Octanal	60	Lifshitz et al. 1970
∝ pinene	1,600	Shaw
Trans-2-hexenal	9,000	Shaw

evaluation are shown in Table 32. Certain conclusions could be stated from this table:

a. Trans-2-hexenal used alone or in combination with other compounds resulted in juices receiving low ratings.

b. The ratings for the pump out juice, introduced as unknown sample, ranged from 4.2 to 4.4 (neither similar or dissimilar to reference juice to slightly similar to reference juice).

c. The reference juice, introduced as unknown sample, received ratings of 6.0, 6.1 and 6.2 (moderately similar to exactly similar to reference juice).

d. The highest ratings were obtained when limonene, citral and either ethyl butyrate or acetaldehyde were added to pump out juice (ratings ranged from slightly similar to moderately similar to reference juice).

4. A trained panel of 11 judges was used to compare the flavor of selected compounds in pump out orange juice to that of reference orange juice.

Acidity of both reference and pump out orange juice were adjusted, by the addition of citric acid, to obtain Brix:acid ratio close to 15:1. The acidities and degrees Brix for both types of juice before and after adjustments are shown in Table 33.

Compounds were added to pump out orange juice at concentrations similar to those found in good quality orange juice. These concentrations are shown in Table 31.

Two types of panels were used:

A. Three samples and a reference juice were presented to the panelists Panelists were asked to smell and taste each sample and compare its flavor to that of the reference juice, and present their ratings on a

Table 32. Sensory ratings of pure compounds added to pumpout, pumpout and reference orange juice.

Compound	Sample	Pumpout Sample	Reference Sample
Acetaldehyde	3.9	3.9	
∝-pinene	4.1		
Citral	4.0	3.9	
Citronellal	4.0		
Dodecanal	4.0		
d-limonene	3.5		
Ethyl butyrate	4.1	3.9	
Linalool	3.8		
Myrcene	2.8		6.2
Nonanal	3.9		6.2
Octanal	4.0		
Trans-2-hexenal (9000ppb)	1.7		
(4500ppb)	1.7		
(3000ppb)	2.3		
Acetaldehyde and citral	2.4		
Acetaldehyde and dodecanal	4.5		
Acetaldehyde and ethyl butyrate	3.7	4.2	
Acetaldehyde and limonene	4.0		
Acetaldehyde and linalool	4.3		
Acetaldehyde and octanal	4.2		
Acetaldehyde and ∝-pinene	4.4		
Ethyl butyrate and linalool	4.5		
Ethyl butyrate and ∝-pinene	4.1	4.2	5.7
Limonene and ∝-pinene	3.6		
Limonene and octanal	3.3		
Linalool and octanal	4.4		
Citral and dodecanal	4.1		
Citral and ethyl butyrate	4.6		
Citral and octanal	4.2		
Citral and ∝-pinene	4.1		5.7
Trans-2-hexenal (3000ppb) and acetaldehyde	1.7		
Trans-2-hexenal (3000ppb) and ethyl butyrate	1.9		

Table 32 (continued)

Compound	Sample	Pumpout Sample	Reference Sample
Trans-2-hexenal (3000ppb) and limonene	2.2		
Trans-2-hexenal (3000ppb) linalool	1.6		
Trans-2-hexenal (3000ppb) and octanal	1.8		
∝-pinene, citral, ethyl butyrate acetaldehyde	4.6		
Limonene, ∝-pinene, octanal	4.0		
Limonene, citral, ethyl butyrate acetaldehyde, ∝-pinene	4.3		
Limonene, acetaldehyde, ethyl butyrate, octanal	4.6		
Limonene, acetaldehyde, octanal	4.2		
Limonene, ethyl butyrate, octanal	4.5		
		4.4	6.1, 6.0
Limonene, citral, acetaldehyde	5.1		
Limonene, citral, ethyl butyrate	5.2		
Limonene, citral, ethyl butyrate, acetaldehyde	5.0		

Table 33. Degrees Brix, percent acidity and Brix:acid ratios of reference and pumpout orange juice.

		Reference Orange Juice					Pumpout			
		ACIDITY		B/A RATIO			ACIDITY		B/A RATIO	
Date	Brix	Before Adjustment	After Adjustment	Before Adjustment	After Adjustment	Brix	Before Adjustment	After Adjustment	Before Adjustment	After Adjustment
1-2-73	12.21	.568	.816	16.20	15.00	13.01	.839	.845	15.50	15.40
1-3-73	12.26	.764	.825	16.05	14.85					
1-8-73	13.04	.780	.842	16.72	15.48					
1-9-73						13.47	.798	.897	16.89	15.02
1-10-73						13.71	.817	.890	16.77	15.40
1-11-73	12.87	.783	.864	16.43	14.89					
1-12-73	12.94	.834	.867	15.52	14.93	13.54	.794	.913	17.05	14.83
1-18-73	12.94	.801	.841	16.16	15.39					
1-19-73						14.41	.758	.926	17.68	14.43
1-22-73	12.76	.791	.831	16.13	15.36	13.88	.841	.923	16.51	15.04
1-24-73						13.68	.817	.910	16.74	15.40
1-30-73						13.26	.788	.874	16.83	15.16
1-31-73	12.91	.811	.870	15.92	14.84	12.71	.768	.851	16.55	14.90
2-2-73						13.53	.810	.939	16.70	14.40
2-5-73						13.38	.796	.897	16.81	14.92
2-7-73	13.34	.807	.860	16.35	15.51					
2-8-73						13.94	.817	.936	17.05	14.89
2-9-73						13.08	.767	.849	17.05	15.30

		Reference Orange Juice					Pumpout			
		ACIDITY		B/A RATIO			ACIDITY		B/A RATIO	
Date	Brix	Before Adjustment	After Adjustment	Before Adjustment	After Adjustment	Brix	Before Adjustment	After Adjustment	Before Adjustment	After Adjustment
2-13-73						13.37	.778	.874	17.19	15.30
2-15-73	12.46	.750	.818	16.62	15.20					
2-16-73						13.24	.753	.875	17.59	15.13
2-19-73	12.78	.743	.837	17.19	15.30	12.71	.729	.828	17.43	15.35
2-19-73						13.41	.756	.872	17.74	15.38
2-20-73	12.72	.745	.840	17.08	15.14	13.46	.743	.896	18.11	15.02
2-22-73						13.16	.784	.861	16.79	15.29
3- 1-73						13.41	.760	.894	17.60	15.00
3- 2-73						12.10	.759	.890	17.83	15.20
3- 5-73	12.76	.753	.834	16.95	15.30					
3- 6-73						13.23	.743	.875	17.80	15.12
3- 8-73						13.03	.740	.853	17.61	15.27
3-12-73						13.43	.775	.891	17.33	15.08
3-13-73	12.56	.737	.840	17.04	14.95					

scale ranging from 7 for exactly like reference juice to 1 for extremely dissimilar to reference juice. Adams and Birdseye orange juice were used as reference juice. Reference juice was occasionally introduced as unknown sample to the panelists. Ratings assigned to the reference juice (as unknown sample) were used to adjust the sample ratings on the basis that reference juice should have received the maximum rating of 7. For example, Adams' reference juice received a rating of 5.9 (Table 34) and to adjust this value to 7.0, a correction factor of 1.186 was used. Ratings for all the samples were adjusted according to this factor. In addition, the adjusted ratings are presented as percent of the maximum rating of 7.

Certain conclusions could be drawn from the results shown in Tables 3, 4 and 5:

1. Adams and Birdseye orange juice received similar ratings, 5.9 and 6.0, respectively (Table 34).
2. The presence of decanal or the absence of d-limonene resulted in lowering the ratings (Table 34).
3. Mixtures containing d-limonene, ethyl butyrate or acetaldehyde, citral and α-pinene received higher ratings than other combinations (Tables 34,35).
4. Pump out orange juice containing 0.02% of the synthetic orange oil mixture received lower ratings than either the reference juice or the pump out juice (Table 36).

B. Three samples and two reference juices were presented. The two reference juices consisted of a pump out juice assigned a rating of 1 and Birdseye juice assigned a rating of 10. Panelists were asked to smell and taste each sample and assign a rating for its flavor

Table 34. Flavor ratings of the modified pumpout orange juice with Adams orange juice as the reference juice. Rating scale 1 to 7 and correction factor 1.186.

Compounds	Ratings		
	Actual	Adjusted	% of Max.
d-Limonene, citral, ∝-pinene	5.0	5.9	84
d-Limonene, citral, ethyl butyrate, acetaldehyde, ∝-pinene	4.9	5.8	83
d-Limonene, citral, ethyl butyrate, acetaldehyde	4.5	5.3	76
d-Limonene, citral, ethyl butyrate, decanal	3.8	4.5	64
d-Limonene, ethyl butyrate, acetaldehyde	4.8	5.7	81
d-Limonene, ∝-pinene, ethyl butyrate	5.0	5.9	84
d-Limonene, citral, ethyl butyrate	5.3	6.3	90
d-Limonene, citral, ehtyl butyrate, acetaldehyde, linalool	5.0	5.9	84
Citral, ethyl butyrate, acetaldehyde	4.4	5.2	74
Adams orange juice	5.9	7.0	100
Birdseye orange juice	6.0	7.0	100

Table 35. Flavor ratings of modified pumpout orange juice with Birdseye orange juice as the reference juice. Rating scale 1 to 7 and correction factor 1.148.

Compounds	Ratings		
	Actual	Adjusted	% of Max.
Birdseye orange juice	6.1	7.0	100
d-Limonene, acetaldehyde, ∝ -pinene, citral	4.0, 4.3	4.6, 4.9	66, 70
d-Limonene, ∝ -pinene, citronellal	3.3	3.8	54
d-Limonene, citral, ethyl butyrate, decanal	3.1	3.6	51
d-Limonene, citronellal, decanal	3.3	3.8	54
d-Limonene, citral, ethyl butyrate, acetaldehyde	3.9, 3.5	4.5, 4.0	64, 57

Table 36. Composition of synthetic orange oil mixture and flavor ratings of modified pumpout orange juice with Birdseye orange juice as the reference juice. Rating scale 1 to 7 and correction factor 1.129.

COMPOSITION

Compound	% of total oil
1. d-Limonene	95.24
2. Myrcene	2.43
3. Valencene	.08
4. β-Caryophyllene	.02
5. Dodecanal	.09
6. Perillaldehyde	.03
7. Carvone	.03
8. Decanal	.61
9. ∝ -Pinene	.40
10. Linalool	.67
11. Citronellal	.13
12. Neral	.06
Geranial	.14
13. β-Sinensal	.03
∝-Sinensal	.02

RATING

Compound	Actual	Adjusted	% of Max.
Birdseye orange juice	6.2	7.0	100
Synthetic orange oil mixture (0.02%)	3.9	4.4	63
Pumpout	4.6	5.2	74

as compared to the two known samples. The good quality reference juice was presented occasionally as unknown sample. Ratings were adjusted as mentioned for panel type A. Results are shown in Tables 37 and 38. Some conclusions are evident:

1. The addition of acetaldehyde to the synthetic orange oil mixture did not result in a noticeable improvement in its rating (Table 37).
2. Pump out orange juice received a rating of 2.5 while Birdseye orange juice received the rating of 8.4 (Table 37).
3. Mixtures of compounds containing acetaldehyde, citral, ethyl butyrate, d-limonene, linalool, octanal and α-pinene added to pump out orange juice resulted in improving the rating of pump out juice from 30% (Table 6) to 75-83% of maximum rating (Table 38).
4. The highest ratings were obtained for the mixtures of: Acetaldehyde, citral, ethyl butyrate and d-limonene (81%); citral, ethyl butyrate and d-limonene (79%); citral, ethyl butyrate, d-limonene and α-pinene(82%); and ethyl butyrate, d-limonene and nonanal (83%) (Table 38).
5. Addition of either of decanal, citronellal or trans-2-hexanal lowered the ratings of the modified pump out juice (Table 38).
6. The mixture containing d-limonene, linalool and α-pinene received a rating of 6.7. Substitution of α-pinene by decanal lowered the rating to 4.5. Mixture containing citral, d-limonene and ethyl butyrate received a rating of 7.9. Substitution of ethyl butyrate by citronellal lowered the rating to 6.3. The lowest rating was obtained as the mixture contained trans-2-hexanal (Table 38).

Table 37. Flavor rating of Birdseye and pumpout orange juice and pumpout orange modified by the synthetic orange oil mixture (0.02%). Rating scale 1 to 10 and correction factor 1.190.

Compounds	Ratings		
	Actual	Adjusted	% of Max.
Birdseye orange juice	8.4	10.0	100
Pumpout	2.5	3.0	30
Synthetic orange oil mixture (0.02%) plus acetaldehyde	3.9	4.6	46

Table 38. Flavor ratings of modified pumpout juice using Birdseye and pumpout orange juice as references. Rating scale 1 to 10 and correction factor 1.321.

Compounds	Rating		
	Actual	Adjusted	% of Max.
Birdseye orange juice	7.6	10.0	100
Acetaldehyde, citral, d-limonene	5.0	6.6	66
Acetaldehyde, citral, linalool	5.1	6.7	67
Acetaldehyde, citral, octanal	3.9	5.2	52
Acetaldehyde, citral, ∝-pinene	4.0	5.3	53
Acetaldehyde, citral, d-limonene, ∝-pinene	4.7	6.2	62
Acetaldehyde, citral, ethyl butyrate, d-limonene	4.3	5.7	57
Acetaldehyde, ethyl butyrate, d-limonene, octanal	5.6,4.9,5.7	7.4,6.5,7.5	74,65,75
Acetaldehyde, d-limonene, octanal	5.1	6.7	67
	5.1	5.1	67
Acetaldehyde, ethyl butyrate, citral d-limonene, octanal	5.1	6.7	67
Acetaldehyde, citral, citronellal, ethyl butyrate, d-limonene, octanal	4.2	5.5	55
Acetaldehyde, citral, citronellal, ethyl butyrate, d-limonene, linalool, octanal, ∝-pinene	5.7	7.5	75
Acetaldehyde, citral, citronellal, decanal, ethyl butyrate, d-limonene, linalool myrcene, nonanal, octanal, ∝-pinene, trans-2-hexanal	1.1	1.5	15
Acetaldehyde, citronellal, d-limonene, linalool, ∝-pinene	5.3	7.0	70
Acetaldehyde, citronellal, ∝-pinene	4.4	5.8	58
Acetaldehyde, citral, citronellal, decanal, ethyl butyrate, d-limonene, linalool, octanal, ∝-pinene	3.4	4.5	45
Acetaldehyde, decanal, d-limonene	4.2	5.5	55
Acetaldehyde, citral, decanal	4.0	5.3	53
Acetaldehyde, d-limonene, ∝-pinene	5.5	7.3	73
	5.1	6.7	67
Acetaldehyde, citral, ethyl butyrate	5.7	7.5	75
Acetaldehyde, d-limonene, nonanal	4.9	6.5	65
Acetaldehyde, d-limonene, linalool	5.2	6.9	69
Acetaldehyde, citronellal, d-limonene	4.8	6.3	63
Acetaldehyde, citral, citronellal	4.6	6.1	61
Acetaldehyde, citral, ethyl butyrate, d-limonene	6.1	8.1	81
Citral, ethyl butyrate, d-limonene	6.0,6.0	7.9,7.9	79,79
Citral, d-limonene, ∝-pinene	4.8	6.3	63
Citral, d-limonene, octanal	5.3	7.0	70
Citral, d-limonene, citronellal	4.8	6.3	63
Citral, ethyl butyrate, octanal	4.8	6.3	63
Citral, ethyl butyrate, d-limonene, octanal, ∝-pinene	5.0	6.6	66
Citral, linalool, octanal	4.9	6.5	65
Citral, citronellal, ethyl butyrate	5.5	7.3	73

Table 38 (continued)

Compounds	Rating		
	Actual	Adjusted	% of Max.
Citral, d-limonene, nonanal	5.1	6.7	67
Citral, decanal, d-limonene	3.5	4.6	46
Citral, octanal, ∝ -pinene	4.8	6.3	63
Citral, d-limonene, linalool	5.7	7.5	75
Citral, citronellal, octanal	4.6	6.1	61
Citral, decanal, linalool	5.4	7.1	71
Citral, ethyl butyrate, linalool	5.8	7.7	77
Citral, ethyl butyrate, ∝ -pinene	5.5	7.3	73
Citral, ethyl butyrate, d-limonene, linalool	5.7	7.5	75
Citral, ethyl butyrate, d-limonene, octanal	5.8	7.5	75
Citral, ethyl butyrate, d-limonene, ∝ -pinene	6.2	8.2	82
	5.0	6.6	66
Citral, citronellal, ethyl butyrate, d-limonene	5.8	7.7	77
Ethyl butyrate, d-limonene, octanal	5.6,5.5	7.4,7.3	74,73
	4.9,6.0	6.5,7.9	65,79
Ethyl butyrate, citronellal, d-limonene	5.1	6.7	67
Ethyl butyrate, d-limonene, linalool	5.6,5.9	7.4,7.8	74,78
Ethyl butyrate, d-limonene, decanal	3.8	5.0	50
Ethyl butyrate, d-limonene, nonanal	6.3	8.3	83
d-limonene, citronellal, linalool	4.7	6.2	62
d-limonene, citronellal, octanal	4.7	6.2	62
d-limonene, octanal, ∝ -pinene	5.2	6.9	69
d-limonene, citronellal, nonanal	4.7	6.2	62
d-limonene, octanal, linalool	4.7	6.2	62
d-limonene, decanal, ∝ -pinene	3.1	4.1	41
d-limonene, linalool, ∝ -pinene	5.1	6.7	67
d-limonene, decanal, linalool	3.4	4.5	45
d-limonene, nonanal, ∝ -pinene	5.1	6.7	67
d-limonene, citronellal, linalool, octanal	5.1	6.7	67
d-limonene, citronellal, linalool, ∝ -pinene	4.9	6.5	65

5. Sensory evaluation methods were used to compare the flavor of selected orange oil and essence components added to pump out orange juice to that of reference (good quality) orange juice.

Acidities of both reference and pump out orange juices were adjusted, by the addition of citric acid, to obtain Brix:acid ratios close to 15:1. The B/A ratios ranged from 14.8:1 to 15.4:1. Orange oil and essence components were added to pump out orange juice at concentrations suggested by Kirchner and Miller (1957), Lifshitz et al. (1970), Shaw and Coleman (1973) and Stanley et al. (1961).

Orange juice samples were served to the panelists slightly chilled at temperatures close to 50°F. Three types of panels were used:

1. Trained panel

Eleven judges (6 males and 5 females) ranging in age from 25 to 55 years old evaluated the flavor of juice samples according to two different methods:

A. Three modified pump out orange juice samples and a reference juice sample were presented to the panelists. Panelists were asked to smell and taste each sample, compare its flavor to that of the reference juice and present their ratings on a scale ranging from 7 for exactly like reference juice to 1 for extremely dissimilar to reference juice. Adams and Birdseye orange juice were used as the good quality reference juices. Reference juice was occasionally introduced as unknown sample to the panelists. Ratings assigned to the reference juice (as unknown sample) were used to adjust pump out sample ratings on the basis that reference juice should have received the maximum rating of 7. The adjusted ratings were calculated as percents of the maximum rating of 7.

Results obtained could be summarized as follows:

a. Adams and Birdseye orange juice (as unknown samples) received similar ratings, 5.9 and 6.0, respectively.

b. Pump out orange juice containing component mixtures of citral, ethyl butyrate and d-limonene or acetaldehyde, citral, ethyl butyrate, d-limonene and α-pinene or ethyl butyrate, d-limonene and α-pinene received higher ratings (83 to 90% of maximum ratings) than other combinations.

c. The absence of d-limonene or the presence of decanal resulted in lowering the ratings by 10-20%.

B. Three modified pump out samples and two reference juices were presented to the panelists. The two reference juices consisted of a pump out juice assigned a rating of 1 and Birdseye juice assigned a rating of 10. Panelists were asked to smell and taste each sample and assign a rating for its flavor as compared to the two known reference samples. The reference juices were presented occasionally as unknown samples. Ratings were adjusted as mentioned previously. Panel responses could be summarized as follows:

a. Pump out orange juice received a rating of 2.5 (30% of maximum rating) while Birdseye orange juice received the rating of 8.4 (100% of maximum).

b. Mixtures of compounds containing acetaldehyde, citral, ethyl butyrate, d-limonene, nonanal, octanal and α-pinene added to pump out orange juice resulted in improving the rating of pump out juice from 30% to 75-83% of maximum rating.

c. The highest ratings were obtained from the mixtures of: citral, ethyl butyrate and d-limonene (79%); acetaldehyde, citral, ethyl butyrate and d-limonene (81%); citral, ethyl butyrate, d-limonene and ∝-pinene (82%); and ethyl butyrate, d-limonene and nonanal (83%).

d. Addition of either decanal, citronellal or trans-2-hexanal lowered the ratings of the modified pump out juice.

2. Expert panel

Orange juice samples were presented to expert panels (12 members at the U.S. Department of Agriculture and Subtropical Products Laboratory, Winter Haven, Florida and 14 members at the University of Florida Research and Education Center at Lake Alfred, Florida). Method of sample presentation was similar to that of method B of the trained panel study. The highest percent maximum ratings were assigned to pump out juice containing acetaldehyde, citral, ethyl butyrate, d-limonene and octanal (78%); acetaldehyde, citral, ethyl butyrate and d-limonene (75%); and acetaldehyde, citral, ethyl butyrate, d-limonene and ∝-pinene (70%). In addition, the expert panel at Lake Alfred rated the orange juice samples on a hedonic scale. The ratings ranged from 3 to 8 but the average rating scores for the modified pump out juices were similar to that of good quality reference juice (Birdseye).

3. Untrained panel

One hundred and five untrained panelists ranging in age from 20 to 60 years old and representing different sexes and races were screened for their ability to indicate if the flavor of pump out juice containing d-limonene or pump out juice containing d-limonene,

acetaldehyde, citral and ethyl butyrate closely resembled the flavor of good quality orange juice (Birdseye). Sixty two panelists were capable of choosing the latter juice over the former. Two samples were of pump out orange juice containing different combinations of the selected components and a reference juice (Birdseye) were presented to the panelists. Panelists were asked to indicate which modified pump out juice was closer in flavor to the reference juice. Five sets of panels were conducted using different combinations of selected components. Samples receiving the highest percentages of selection as closely resembling reference orange juice were pump out juice containing acetaldehyde, ethyl butyrate, d-limonene and octanal; citral, ethyl butyrate, d-limonene and α-pinene; and acetaldehyde, citral, ethyl butyrate, d-limonene and octanal. It seems that pump out juice containing acetaldehyde, citral, ethyl butyrate, d-limonene and octanal received the highest ratings by the three methods of sensory panels used.

SUMMARY AND CONCLUSIONS

The flavor and odor threshold concentrations for selected volatile components of orange juice were evaluated. The influence of selected non-volatile components on the flavor threshold of d-limonene and 5' nucleotides on the flavor threshold of octanal were determined. An examination of some of the variables present in a sensory evaluation panel were also made. Within the limitations of this study the following conclusions were made:

1. The following values (ppb) for flavor and odor thresholds, respectively, were determined: ethyl butyrate, 0.13, 0.12; methyl butyrate, 59, 58; ethyl propionate, 4.9, 9.9; n-octyl acetate, 205, 48; n-nonyl acetate, 266, 57; octanol, 54, 186, decanol, 23, 47; dodecanol, 66, 74; α-terpineol, 323, 284, linalool, 3.8, 5.3; d-limonene, 214, 60; d-carvone 86, 2.7; p-cymene 13.3, 11.4; Ethyl vinyl ketone 1.2, 0.9; ∠-pinene 1014, 9.5; ethanal 22.0, 17.0; butanal 5.26, 15.9; hexanal 3.66, 9.18; octanal 0.52, 1.41; nonanal 4.25, 2.53; decanal 3.02, 1.97; dodecanal 1.07, 0.53; trans-2-hexenal 49.3, 24.2; citral 41.4, 85.3; perillaldehyde 25.3, 30.1.

2. d-Limonene, linalool and decanol may have a direct effect on the flavor of orange juice. The effect of octanol and α-terpineol may be questioned. Concentrations for the other compounds have not been reported for use in determining flavor significance.

3. A possible relationship between odor threshold and size of the molecule was indicated for alcohols and esters. The threshold appeared to decrease with increasing size until an optimun size was reached, where the threshold then increased with increasing size.

4. Since 5'-nucleotides have recently been found in relatively abundant quantities in orange juice, their influence on the flavor

threshold of octanal was determined. Ten screened and trained panelists evaluated five replications of octanal in six aqueous solutions of AMP, ADP, ATP, GMP, GDP, GTP, and in distilled water by paired comparison tests. Nucleotides were presented in solutions at 10 ppm, their approximate concentrations in fresh juice. The flavor threshold of octanal in distilled water was 1.38 ppb. Thresholds for octanal in solutions containing ADP and GMP were significantly lower at 0.85 and 0.86 ppb, respectively.

5. The presence of KCl or MSG did not seem to increase the percent detection of octanal.

6. The presence of sugar and pectin at concentrations approximating those in orange juice did not significantly influence the flavor threshold of d-limonene.

7. There were significant variations among panelists in their abilities to detect small concentrations and to judge differences accurately.

8. There were no significant differences in panel responses to replications on the different days of the week.

9. The accuracy of the panelists in detecting blanks was influenced significantly by solvents tested which decreased correctness in response with the exception of the solution containing only sugar. The solution containing only acid decreased the correctness in response the most followed by pectin alone and the combinations.

10. There was a significant increase in the number of incorrect responses to the blanks placed next to higher concentrations, which was possibly caused by carryover effects or panelist anticipation of strong concentrations.

11. Sensory evaluations of the flavor of pump out orange juice

as influenced by the addition of selected orange oil and essence components indicated that the highest ratings were assigned to pump out orange juice containing mixtures of 4 to 6 compounds depending on the type of panel used. The expert panel assigned the highest rating to the mixture of acetaldehyde, citral, ethyl butyrate, d-limonene and octanal; the trained panel assigned the highest rating to the mixture of citral, ethyl butyrate, d-limonene and α-pinene or the mixture of acetaldehyde, citral, ethyl butyrate and d-limonene. The untrained panel (a small scale consumer panel) offered the highest flavor ratings to pump out juice fortified with the mixtures acetaldehyde, ethyl butyrate, d-limonene and octanal; citral, ethyl butyrate, d-limonene, and α-pinene; or acetaldehyde, citral, ethyl butyrate, d-limonene and octanal.

LIST OF REFERENCES

Amerine, M. A., R. M. Pangborn, and E. B. Roessler. 1965. Principles of Sensory Evaluation of Food. Academic Press, Inc., New York.

Amoore, J. E. 1963. Stereochemical theory of olfaction. Nature 198:271-272.

Amoore, J. E., J. W. Johnston, Jr., and M. Rubin. 1964. The Stereochemical theory of odor. Scientific American 210(12):42-49.

Amoore, J. E. and D. Venstrom. 1966. Sensory analysis of odor qualities in terms of the stereochemical theory. J. Food Sci. 31:118-128.

Attaway, J. A., R. W. Wolford, and G. J. Edwards. 1962. Isolation of some volatile carbonyl components from orange essence. J. Agr. Food Chem. 10:102-104.

Barmore, C. R. 1972. Changes in ribonucleic acids in citrus fruit with growth and development. PhD Dissertation. University of Florida, Gainesville, Florida.

Baten, W. D. 1950. Reaction of age groups to organoleptic tests. Food Technol. 4:277-279.

Beidler, L. M. 1966. A physiological basis of taste sensation. J. Food Sci. 31:275-280.

Bennett, G., Liska, B. J. and W. L. Hempenius. 1965. Effect of other flavor components on the perception of diacetyl in fermented dairy products. J. Food Sci. 30:35.

Bennett, T. P. and E. Freidan. 1966. Modern topics in biochemistry structure and function of biological molecules. The MacMillan Co., New York.

Berg, H. W., F. Filipello, E. Hinreiner, and A. D. Webb. 1955a. Evaluation of thresholds and minimum difference concentrations for various constituents of wines. Food Technol. 9:23-26.

Berg, H. W., Filipello, F., Hinreiner, E. and Webb, A. D. 1955b. Evaluation of thresholds and minimum difference concentrations for various constituents of wines. II. Sweetness: The effect of ethyl alcohol, organic acids and Tannin. Food Technol. 9:138.

Bernhard, R. A. 1960. Stationary liquid phases for use in gas-liquid chromatography suitable for the separation of the components of essential oils. Food Research 25:531-537.

Blair, J. S., E. M. Godar, J. E. Masters, and D. W. Riester. 1952. Exploratory experiments to identify chemical reactions causing flavor deterioration during storage of canned orange juice. I. Incompatibility of peel-oil constituents with the acid juice. Food Research 17:235.

Blakeslee, A. F. and M. R. Salmon. 1931. Odor and taste blindness. Eugenical News 16:105.

Braverman, J. B. S. 1949. "Citrus Products," Interscience Publishers, LTD., London.

Bruemmer, J. H. 1969. Redox state of nicotinamide-adenine dinucleotides in citrus fruit. J. Food Chem. 17:1312-1315.

Buttery, R. G., L. C. Ling and D. G. Guadagni. 1969. Volatiles of aldehydes, ketones, and esters in dilute water solutions. J. Agri. Food Chem. 17:385-389.

Buttery, R. G., R. M. Seifert, D. G. Guadagni and L. C. Ling. 1971. Characterization of additional volatile components of tomato. J. Agr. Food Chem. 19:524-528.

Coleman, R. L., E. D. Lund and M. G. Moshonos. 1969. A research note composition of orange essence oil. J. Food Sci. 34:610-611.

Crocker, E. C. 1945. "Flavor," McGraw Hill Book Co., New York.

Day, E. A., D. A. Lillard, and M. W. Montogomery. 1963. Autoxidation of milk lipids III. Effect on flavor of the additive interactions of carbonyl compounds at subthreshold concentrations. J. Dairy Sci. 46:291-294.

Dinsmore, H. L. and S. Negy. 1971. A rapid gas chromatographic method for studying volatile carbonyl compounds from orange juice and their changes during storage. J. Agr. Food Chem. 19:517-519.

Fabian, F. W. and Blum, H. B. 1943. Relative taste potency of some basic food constituents and their competitive and compensatory action. Food Res. 8:179.

Fisher, R. and F. Griffin. 1963. Quinine dimorphism: a cardinal determinant of taste sensitivity. Nature 200:343-347.

Flath, R. A., D. R. Black, D. G. Guadagni, W. H. McFadden and T. H. Schultz. 1967. Identification and organoleptic evaluation of compounds in Delicious apple essence. J. Food Chem. 15:29-30.

Florida Citrus Mutual. 1969. Annual Statistical Report, 1968-69 Season.

Forss, D. A. 1969. Role of lipids in flavors. J. Agr. Food Chem. 17: 681-685.

Furukawa, S. and Dirnizawa. 1920. Essential oil of Perylla nankinensis, Dene. J. Chem. Ind. Tokyo 23:242-263 (Chem. Abstr. 14:2839, 1920).

Gregson, R. A. 1962. A rating-scale method for determining absolute taste threshold. J. Food Sci. 27:376-380.

Guadagni, D. G., R. G. Buttery and S. Okano. 1963a. Odor thresholds of some organic compounds associated with food flavors. J. Sci. Fd. Agri. 14: 761-765.

Guadagni, D. G., R. G. Buttery, S. Okano, and H. K. Burr. 1963b. Additive effect of sub-threshold concentrations of some organic compounds associated with food aromas. Nature 200:1288-1289.

Hall, J. A. and C. P. Wilson. 1925. The volatile constituents of Valencia orange juice. J. Am. Chem. Soc. 47:2575-2584.

Halsey, W. D. (Ed). 1964. Collier's Encyclopedia, Vol. 18, pp 576-578. The Crowell-Collier Publishing Company, New York.

Harrison, S. and L. W. Elder. 1950. Some applications of statistics laboratory taste testing. Food Technol. 4:434-439.

Hashida, W., T. Mouri, and J. Shiga. 1966. Applications of 5'-ribonucleotides to canned vegetables. Food Technol. 20:945-973.

Hinreiner, E., F. Filipello, A. D. Webb and H. W. Berg. 1955a. Evaluation of thresholds and minimum difference concentrations for various constituents of wines. III. Ethyl alcohol, glycerol and acidity in aqueous solution. Food Technol. 9:351-353.

Hinreiner, E., F. Filipello, H. W. Berg and A. D. Webb. 1955b. Evaluation of threshold and minimum difference concentrations for various constituents of wines. IV. Detectable differences in wine. Food Technol. 9:489-490.

Hodges, A. M. 1962. The influence of environmental variables on the absolute threshold of taste perception. MSA, University of Florida, Gainesville.

Jones, N. R. 1969. Significance of ribomononucleotides and their metabolites. J. Agr. Food Chem. 17:712-716.

Kalckar, H. M. 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. J. Biol. Chem. 169:445-459.

Kamen, J. M., F. J. Pilgrim, Gutman and B. J. Kroll. 1961. Interactions of suprathreshold taste stimuli. J. Exptl. Psychol. 62:348.

Kefford, J. F. 1959. The chemical constituents of citrus fruits. In Advances in Food Research. Academic Press, New York. Vol. IX, pp. 285-372.

Keith, E. S. and J. J. Powers. 1968. Determination of flavor threshold levels and sub-threshold, additive, and concentration effects. J. Food Sci. 33:213-218.

Kesterson, J. W. and R. Hendrickson. 1953. Essential oils from Florida citrus. Florida Agr. Exp. Tech. Bull. 521:1-70.

King, F. B. 1937. Obtaining a panel for judging flavor in foods. Food Research 2:207-219.

Kirchner, J. G. 1961. Oils in peel, juice sac, and seed. In The orange. The University of California Printing Department, California. pp. 265-301.

Kirchner, J. G. and J. M. Miller. 1957. Volatile water-soluble and oil constituents of Valencia orange juice. J. Agr. Food Chem. 5:283-291.

Klopping, H. L. 1971. Olfactory theories and the odors of small molecules. J. Agr. Food Chem. 19:999-1003.

Kramer, A., E. F. Murphy, A. M. Briant, M. Wang and M. E. Kirkpatrick. 1961. Studies in taste panel methodology. J. Agr. Food Chem. 9:224-228.

Kuninaka, A. 1960. Studies on the taste of ribonucleic acid derivatives. J. Agr. Soc. Japan 34:489-492.

Kuninaka, A. 1966. Recent studies of 5'-nucleotides as new flavor enhancers. In Flavor Chemistry. American Chemical Society, Washington, D.C.

Kuninaka, A., M. Kibi and K. Sakaguchi. 1964. History & development of flavor nucleotides. Food Technol. 18:287-293.

Kurtzman, C. H. and L. B. Sjostrom. 1964. The flavor-modifying properties of disodium inosenate. Food Technol. 18:1467-1469.

Laffort, P. 1963. Essai do standardisation des seuils olfactifs humains pour 192 corps purs. Arch. Sci. Physiol. 17:75-105.

Lane, E. A., N. H. Ishler and G. A. Bullman. 1954. Reliability of taste testing and consumer testing methods. In Fatigue in taste testing. Food Technol. 8:387-388.

Langler, J. E. and E. A. Day. 1964. Development and flavor properties of methyl ketones in milk fat. J. Dairy Sci. 47:1291.

Lea, C. H. and P. A. Swoboda. 1958. The flavour of aliphatic aldehydes. Chem. & Ind. 1289-1290.

Lifshitz, A., W. L. Stanley and Y. Stepak. 1970. Comparison of Valencia essential oil from California, Florida and Israel. J. Food Sci. 35:547-548.

Luh, B. S. and K. C. Chen. 1969. Acid-soluble ribonucleotides in fruits and vegetables. Fruchtsaft-Industrie 14:146-162.

Lund, E. D., C. J. Wagner, Jr., and R. E. Berg. 1971. Quantitative analyses of aroma solutions and concentrations. Paper presented at Conference on Citrus Chemistry and Utilization. October 8, 1971. Winter Haven, Florida.

Mackey, A. O. and K. Valassi. 1956. The discernment of primary tastes in the presence of different food textures. Food Technol. 10:238.

McNamara, B. P. and W. H. Danker. 1968. Odor and taste. In "Basic Principles of Sensory Evaluation," American Society for Testing and Materials, pp. 13-18. Philadelphia, Pa.

Meijboom, P. W. 1964. Relationship between molecular structure and flavor perceptibility of aliphatic aldehydes. J. Am. Oil Chemists' Soc. 41: 326-328.

Minich, S. K., G. J. Mountney, and L. Prudent. 1966. The effect of age and sex of panel members on taste thresholds for standard chicken broth. Food Technol. 20:970-973.

Mitchell, J. W. 1957a. Problems in taste difference testing. I. Test environment. Food Technol. II:476-477.

Moncrieff, R. W. 1967. Introduction to the symposium. In "The Chemistry and Physiology of Flavors," eds. Shultz, H. W., Day, E. A. and Libbey, L. M. pp. 3-22. AVI Publishing Co., Westport, Conn.

Morrison, R. T. and R. N. Boyd. 1966. Organic Chemistry. Allyn and Bacon, Inc., Boston, MA.

Moshonas, M. G. and E. D. Lund. 1969a. Aldehydes, and esters in Valencia orange peel oil. J. Food Sci. 34:502-503.

Naves, Y. R. 1947. The aldehyde fraction of essential oil of sweet orange. Perfumery Essent. Oil Record 38:295-298, 320.

Ohta, Y. and Y. Hirose. 1966. Constituents of cold-pressed peel oil of Citrus natsudaidai Hayata. Agr. Biol. Chem. 30:1196-1201.

Pangborn, R. M. 1960. Taste interrelationships. Food Research 25:245.

Patton, S. and D. V. Josephson. 1957. A method for determining significance of volatile flavor compounds in foods. Food Research 22:316-318.

Petrus, D. R., M. H. Dougherty and R. W. Wolford. 1970. A quantitative total aldehydes test useful in evaluating and blending citrus essence and concentrated citrus products. J. Agr. Food Chem. 18:908-910.

Rice, R. G., G. J. Keller and E. A. Beavens. 1952. Flavor fortification of California frozen orange concentrate. Food Technol. 6:35-39.

Rouse, A. H. 1971. Personal Communications.

Sagarin, E. 1954. Odor: a proposal for some basic definition. ASTM Spec. Tech. Publ. 164:3-8.

Schinneller, D. J. 1972. Flavor and aroma thresholds of selected aldehydes and interactions with 5'-nucleotides associated with orange juice. M.S.A. Thesis, University of Florida, Gainesville, Florida.

Schultz, T. H., R. Teranishi, W. H. McFadden, P. W. Kilpatrick and J. Corse. 1964. Volatiles from oranges. II. Constituents of the juice identified by mass spectra. J. Food Sci. 29:790-795.

Schutz, H. G. 1971. Sources of invalidity in the sensory evaluation of foods. Food Technol. 25:249-253.

Schwimmer, S. and D. G. Guadagni. 1967. Cysteine induced odor intensification in onions and other foods. J. Food Sci. 32:405-408.

Shaw, P. E. and R. L. Coleman. 1971. Quantitative analysis of a highly volatile fraction from Valencia orange essence oil. J. Agr. Food Chem. 19:1276.

Shaw, P. E. and R. L. Coleman. 1973. Quantitative composition of cold-pressed orange oils. Submitted to J. Agr. Food Chem.

Shimazono, H. 1964. Distribution of 5'-ribonucleotides in foods and their application to foods. Food Technol. 18:294-303.

Shimazono, H. 1965. A new flavour enhancer the 5'-ribonucleotides. Food Manuf. 40(11):53-55.

Sick, T. J., I. A. Albin, L. A. Sather and R. C. Lindsay. 1969. Taste thresholds of butter volatiles in deodorized butteroil medium. J. Food Sci. 34:265-267.

Siek, T. J., Albin, I. A., Sather, L. A. and Lindsay, R. C. 1969. Taste thresholds of butter volatiles in deodorized butteroil medium. J. Food Sci. 34:265.

Sinclair, W. B. 1961. "The Orange--Its Biochemistry and Physiology." University of California, Div. of Agric., Sci. Riverside, Ca.

Snedecor, G. W. and W. G. Cochran. 1967. "Statistical Methods." The Iowa State University Press. Ames, Iowa.

Snyder, L. H. 1931. Inherited taste deficiency. Science 74:151.

Stahl, W. H. 1971. Research and Development Lab, McCormic & Company, Inc., 204 Wight Ave., Cockeysville, Md. Personal Communication.

Stanley, W. L., R. M. Ikeda, S. H. Vannier, and L. A. Rolle. 1961. Determination of the relative concentrations of the major aldehydes in lemon, orange and grapefruit oils by gas chromatography. J. Food Sci.26:43-48.

Stier, E. F., F. M. Sawyer and P. E. Fergenson. 1967. A comparison of methodology used in determining the flavor effect of 5'-ribonucleotides on processed foods. Food Technol. 21:627-1646.

Stone, H., C. S. Ough, and R. M. Pangborn. 1962. J. Food Sci. 27:197-202.

Swets, J. A. 1961. Is there a sensory threshold? Science 134:168-177.

Ting, S. V. and Attaway, J. A. 1971. Citrus fruits. In "The Biochemistry of Fruits and Their Products," ed. A. C. Hulme, Vol. 2, pp. 107-169. Academic Press, London and New York.

Tompkins, M. D. and G. B. Pratt. 1959. Comparison of flavor evaluation methods for frozen citrus concentrate. Food Technol. 13:149-152.

Tressler, D. K., M. A. Joslyn and G. L. Marsh. 1939. "Fruit and Vegetable Juices." Avi Publishing Co., New York.

Tressler, D. K. and M. A. Joslyn. 1961. "Fruit and Vegetable Juice Processing Technology." Avi Publishing Co., Westport, Conn.

Valdes, R. M., Hinreiner, E. and Simone, M. J. 1956. Effect of sucrose and organic acids on apparent flavor intensity. I. Aqueous Solutions. Food Technol. 10:282.

Wagner, J. R., D. S. Titus and J. E. Schade. 1963. New opportunities for flavor modification. Food Technol. 17:730-734.

Weurman, C. 1963. Recent developments in food odour research methods. In Recent Advances in Food Science. Butterworths, London. Vol. 3, pp. 37-150.

Wolford, R. W., G. E. Alberding and J. A. Attaway. 1962. Analysis of recovered natural orange essence by gas chromatography. J. Agr. Food Chem. 10:297-301.

Wolford, R. W., J. A. Attaway, G. E. Alberding and C. D. Atkins. 1963. Analysis of the flavor and aroma of Florida orange juice by gas chromatography. J. Food Sci. 28:320-328.

Wolford, R. W. and J. A. Attaway. 1967. Analysis of recovered natural orange enhancement materials using gas chromatography. J. Agr. Food Chem. 15: 369-377.

Wolford, R. W., M. H. Dougherty and D. R. Petrus. 1969. Citrus juice essence. Scientific Tech. Comm., Int. Fed. of Fruit Juice Processer, May 22-24.

Wolford, R. W., J. W. Kesterson and J. A. Attaway. 1971. Physiochemical properties of citrus essential oils from Florida. J. Agr. Food Chem. 19:1097.

Woskow, M. H. 1969. Selectivity in flavor modification by 5'-ribonucleotides. Food Technol. 23:1364-1369.

Yamaguchi, S. 1967. The synergistic taste effect of monosodium glutamate and disodium 5'-inosenate. J. Food Sci. 32:473-478.

Yamaguchi, S., T. Yoshikawa, S. Ikeda and Ninomiya. 1967. Synergistic taste effects of some new ribonucleotide derivatives. Agr. Biol. Chem. 32:797-802.

Yamaguchi, S., T. Yoshikawa, S. Ikeda and T. Ninomiya. 1971. Measurement of relative taste intensity of some 1-a-amino acids and 5'-nucleotides. J. Food Sci. 36:846-849.

Yensen, R. 1958. Influence of water deprivation on taste sensitivity in man. Nature 182:677-679.

Zotterman, Y. and H. Diamant. 1959. Has water a specific taste? Nature 183(1):191-192.

APPENDICES

APPENDIX A

Data for determination of odor thresholds. Logarithms of aqueous concentrations of aldehydes with corresponding percentages of detection by panelists in parentheses.

Aldehyde	Log of Cencentration in PPB(wt/vol) (Percent of Detection by Panelist)				Mean	Regression Slope
Ethanal	1.00 (28.8)	1.30 (61.5)	1.48 (70.2)	1.70 (79.6)	1.37 (60.0)	72.6
Butanal	0.70 (30.6)	1.00 (43.3)	1.18 (60.3)	1.30 (44.5)	1.04 (44.7)	33.9
Hexanal	0.00 (27.9)	0.47 (22.0)	0.70 (38.9)	1.00 (59.4)	0.54 (37.0)	30.9
Octanal	-0.30 (26.5)	0.00 (45.0)	0.30 (59.0)	0.48 (63.8)	0.12 (48.6)	48.4
Nonanal	0.00 (20.6)	0.30 (44.4)	0.48 (60.2)	0.70 (65.8)	0.37 (47.8)	67.1
Decanal	-0.30 (40.7)	0.00 (36.0)	0.30 (45.1)	0.70 (65.6)	0.17 (46.8)	26.3
Dodecanal	-1.00 (22.9)	-0.30 (36.6)	0.00 (62.3)	0.30 (82.1)	-0.25 (51.0)	44.9
trans-2-Hexenal	1.18 (36.4)	1.30 (47.6)	1.40 (44.5)	1.60 (67.3)	1.37 (49.0)	68.7
Citral	1.78 (34.8)	1.90 (43.1)	2.00 (54.4)	2.08 (72.0)	1.94 (51.1)	120
Perillaldehyde	1.00 (29.2)	1.30 (35.8)	1.48 (43.6)	1.65 (66.0)	1.36 (43.6)	52.5

APPENDIX B

Data for determination of flavor thresholds. Logarithms of aqueous concentrations of aldehydes with corresponding percentages of detection by panelists in parentheses.

Aldehyde	Log of Concentration in PPB (wt/vol) (Percent of Detection by Panelist)				Mean	Regression Slope
Ethanal	1.00 (27.7)	1.30 (49.0)	1.48 (55.4)	1.60 (68.7)	1.37 (50.2)	64.8
Butanal	0.48 (31.1)	0.70 (50.6)	0.85 (63.5)	1.00 (65.4)	0.76 (52.4)	70.6
Hexanal	0.00 (26.9)	0.48 (35.6)	0.70 (51.5)	0.85 (74.3)	0.51 (47.1)	50.4
Octanal	-0.52 (37.8)	-0.30 (49.0)	-0.15 (55.8)	0.00 (65.4)	-0.24 (52.0)	52.1
Nonanal	0.30 (29.4)	0.48 (30.5)	0.60 (47.7)	0.70 (59.2)	0.52 (41.7)	76.8
Decanal	-0.30 (40.7)	0.00 (36.0)	0.30 (45.1)	0.70 (65.6)	0.17 (46.8)	26.4
Dodecanal	-0.30 (33.3)	0.00 (47.2)	0.30 (60.6)	0.48 (78.3)	0.12 (54.8)	55.1
trans-2-Hexenal	1.30 (33.0)	1.60 (38.4)	1.70 (47.4)	1.78 (61.2)	1.60 (45.0)	51.2
Citral	1.30 (33.6)	1.60 (45.6)	1.70 (56.8)	1.78 (59.1)	1.60 (48.8)	54.8
Perillaldehyde	1.00 (26.9)	1.48 (46.1)	1.60 (72.2)	1.70 (64.7)	1.44 (52.5)	60.3

APPENDIX C

Data for determination of flavor threshold of octanal in the presence of selected 5'-Nucleotides. Logarithms of the concentrations of octanal with corresponding percentages of detection by panelists.

5'-Nucleotide	% of Detection for Logs of Conc. in PPB(wt/vol) -1.00	-0.52	0.00	0.48	1.00	Mean -0.01	Regression Slope
Control	6.00	24.0	48.0	64.0	80.0	44.4	37.6
ATP	8.00	18.0	48.0	72.0	84.0	46.0	41.2
GTP	4.00	20.0	50.0	70.0	86.0	46.0	42.8
AMP	6.00	18.0	52.0	72.0	84.0	46.4	42.0
GDP	12.0	14.0	60.0	76.0	88.0	50.0	42.9
GMP	6.00	30.0	60.0	80.0	86.0	52.4	41.9
ADP	14.0	32.0	50.0	74.0	92.0	52.4	39.6

APPENDIX D

Statistical formulae used to compute thresholds by inverselinear regression.

Formulae

X_i = log of concentration of aldehyde in ppb

Y_i = percent of detection

n = number of repetitions

$E \equiv \sum_{i=1}^{n}$ = summation from i=1 to i=n

$$\overline{X} = E(X_i/n)$$

$$\overline{Y} = E(Y_i n)$$

$$E(x_i^2) = E(X_i^2) - (EX_i)^2/n$$

$$E(y_i^2) = E(Y_i^2) - (EY_i)^2/n$$

$$E(x_i y_i) = E(Y_i X_i) - (EX_i)(EY_i)/n$$

$$b = E(x_i y_i)/E(x_i^2)$$

$$\hat{X}_i = \overline{X}_i + (50-\overline{Y}_i)/b$$

$$S_{y.x}^2 = (Ey_i^2 - (Ex_i y_i)^2/Ex_i^2)/(n-2)$$

$$S_b^2 = S_{y.x}^2/Ex_i^2$$

$$V(\hat{x}_i) = (S_{y.x}^2/n)(1/b^2+3S_b^2/b^4+3S_b^4/b^6) + (50-\overline{Y})^2(S_b^2/b^4+2S_b^4/b^6)$$

$$t = (\hat{X}_i-\hat{X}_2)\,\mathrm{SQRT}(V(\hat{x}_1)+V(\hat{x}_2))$$

APPENDIX E

Fortran IV statements for the computation of aldehyde thresholds by inverse linear regression.

Statements

```
1 DIMENSIONX(4),Y(4),Z(4)
2 READ(5,3)(Z(I),I=1,4),(Y(I),I=1,4)
3 FORMAT(8F5.1)
4 A=0.0
5 B=0.0
6 C=0.0
7 D=0.0
8 E=0.0
9 DO15I=1,4
10 X(I)=ALOG10(Z(I))
11 A=A+X(I)
12 B=B+X(I)**2
13 C=C+Y(I)
14 D=D+Y(I)**2
15 E=E+X(I)*Y(I)
16 SX=B-((A**2)/4.0)
17 SY=D-((C**2)/4.0)
18 SXY=E-(A*C)/4.0
19 B=SXY/SX
20 XBAR=A/4.0
21 YBAR=C/4.0
```

APPENDIX E continued

```
22 SXHAT=(5.0-YBAR)/B

23 XHAT=XBAR+SXHAT

24 S2YX=(SY-SXY**2/SX)/2.0

25 S2B=S2YX/SX

26 VAR=((S2YX/4.0)*(1.0/B**2+3.0*S2B/B**4+(3.0*S2B**2)/B**6))+(((50.0-
   YBAR)**2)*(S2B/B**4+2.0*S2B**2/B**6))

27 SIGMA=SQRT(VAR)

28 ZSIGMA=SIGMA*1.96

29 CL95LO=XHAT-ZSIGMA

30 CL95UP=XHAT+ZSIGMA

31 AXBAR=10.0**XBAR

32 AXHAT=10.0**XHAT

33 AVAR=10.0**VAR

34 ASIGMA=10.0**SIGMA

35 AZSIGM=10.0**ZSIGMA

36 ACL95L=10.0**CL95LO

37 ACL96U=10.0**CL95UP

38 WRITE(6,39)(Z(I), I=1,4),(X(I),I=1,4),(Y(I),I=1,4),B,XBAR,YBAR,XHAT
   VAR,SIGMA,ZSIGMA,CL95LO,CL95UP,AXBAR,AVAR,ASIGMA,AZSIGM,AXHAT,
   ACL95L,ACL95U

39 FORMAT('1',28F15.1)

40 GO TO 2

   STOP
```

Concentration values and percents of detection are read into program according to format outlined in statements 2 and 3

APPENDIX F

Fortran IV statements for the computation of flavor thresholds of octanal by inverse linear regression.

Statements

```
 1 DIMENSIONX(5),Y(5,7),N(7),YBAR(7),BB(7),XHAT(7),VXHAT(7),TTEST(7)
 2 X(1)=ALOG10(0.1)
 3 X(2)-ALOG10(0.3)
 4 X(3)=ALOG10(1.0)
 5 X(4)=ALOG10(3.0)
 6 X(5)=ALOG10(10.0)
 7 XBAR=(X(1)+X(2)+X(3)+X(4)+X(5))/5.0
 8 READ(5,9)((Y(J,K),J=1,5),K=1,7)
 9 FORMAT(5F4.1)
10 DO42K=1,7
11 A=0.0
12 B=0.0
13 C=0.0
14 D=0.0
15 E=0.0
16 F=0.0
17DO22J=1,5
18 A=A+X(J)**2
19 B=B+X(J)
20 C=C+Y(J,K)**2
21 D=D+Y(J,K)
22 E=E+X(J)*Y(J,K)
23 SX2=A-B**2/5.0
24 SY2=C-D**2/5,0
25 SXY=E-(B*D)/5.0
26 BB(K)=SXY/SX2
27 YBAR(K)=D/5.0
28 SXHAT=(50.0-YBAR(K))/BB(K)
29 XHAT(K)=XBAR+SXHAT
30 S2YX=(SY2-SXY**2/SX2)/3.0
31 S2B-S2YX/SX2
```

Appendix F (continued)

```
32 VXHAT(K)=((S2YX/5.0)*(1.0/BB(K)**2+3.0*S2B/B(K)**4+(3.0*S2B**2)/
   BB(K)**6))+(((50.0-YBAR(K))**2)*(S2B/BB(K)**4+2.0*s2B**2/BB(K)*2*6))
33 AVXHAT=10.0**VXHAT(K)
34 SVXHAT=SQRT(VXHAR(K))
35 AXHAT=10.0**XHAT(K)
36 VXHATL=10.0**(XHAT(K)-SVXHAT)
37 VXHATU=10.0**(XHAT(K)+SVXHAT)
38 WRITE(6,39)(X(I),I=1,5),XBAR,(Y(J,K)J=1,5),YBAR(K),SX2,SY2,SXY,S2YX,
   S2B,BB(K),XHAT(K)AXHAT,VXHAT(K),AVXHAT
40 WRITE(6,41)VXHATL,VXHATU
42 CONTINUE
43 DO51K=1,6
44 M=7-K
45 DO48J-1,M
46 AMD-ABS(XHAT(K)-XHAT(K+J))
47 AVT-ABS(VXHAT(K)+VXHAT(K+J))
48 TTEST(J)=AMD/SQRT(AVT)
49 WRITE(6,50)K,(TTEST(J),J=i,M)
51 CONTINUE
   STOP
```

---

percents of detection are read into program according to format outlined in Statements 8 and 9.

APPENDIX C

Fortran IV statements for computing analysis of variation in the detection of octanal.

Statements

```
 1 DIMENSIONX(5,5,7),C(5,7),TC(5),R(5,7),TR(5),N(7),Z(5,5,7)
 2 REALNREAD(5,4)(((Z(I,J,K),I=1,5),J=1,5),K=1,7)
 3 FORMAT(5F4.1)
 4 TX=0.0
 5 TX2=0.0
 6 D011K=1,7
 7 D011J=1,5
 8 D011I=1,5
 9 X(I,J,K)=ARSIN(Z(I,J,K)/10.0
10 TX=TX+X(I,J,K,)
11 TX2=TX2+X(I,J,K)**2
12 CF=TX**2/175.0
13 TSS=TX2-CF
14 SR=0.0
15 D021I-1,5
16 B-0.0
17 D019K=1,7
   A=0.0
18 D019J-1.,5
19 A=A+X(I,J,K)
   R(I,J)=A
   B=B+A
20 TR(I)=B
21 SR=SR+B**2
22 SSR=SR/35.0-CF
```

APPENDIX G (continued)

```
23 ST-o.o
24 D030K=1,7
25 D030J=1,5
26 T=0.0
27 D028I=1,5
28 T=T+X(I,J,K)
29 C(J,K)=T
30 ST=ST+T**2
31 SST=ST/5.0-CF
32 SN=0.0
33 D039K=1,7
34 A=0.0
35 D037J=1,5
36 D037I=1,5
37 A=A+X(I,J,K)
38 SN=SN+A**2
39 N(K)=A
40 SSN=SN/25.0-CF
41 SC=0.0
42 D048J=1,5
43 A=0.0
44 D046K=1,7
45 D046I=1,5
46 A=A+X(I,J,K)
47 SC=SC+A**2
48 TC(J)=A
49 SSC=SC/35.0-CF
50 SSC1=(TC(1)*(-2.0)-TC(2)+TC(4)+TC(5)*2.0)**2./350.0
```

APPENDIX G (continued)

```
51 SSC2=(TC(1)*2.0-TC(2)-TC(3)*2.0-TC(4)+TC(5)*2.0)**2/490.0
52 SSC3=(-TC(1)+TC(2)*2.0-TC(4)*2.0+TC(5))**2/350.0
53 SSC4=(TC(1)-TC(2)*4.0+TC(3)*6.0-TC(4)*4.0+TC(5))**2/2450.0
54 SSNXC=SST-SSN-SSC
55 SSE=TSS-SSR-SST
56 XMSR=SSR/4.0
57 XMST=SST/34.0
58 XMSN=SSN/6.0
59 XMSC=SSC/4.0
60 XMSNXC=SSNXC/24.0
61 XMSE=SSE/136.0
62 FREP=XMSR/XMSE
63 FMST=XMST/XMSE
64 FNUC=XMSN/XMSE
65 FCON=XMSC/XMSE
66 FCON1=SSC1/XMSE
67 FCON2=SSC2/XMSE
68 FCON3=SSC3/XMSE
69 FCON4=SSC4/XMSE
70 FNXC=XMSNXC/XMSE
73 DO82K=1,7
76 DO79J=1,5
77 WRITE(6,78)J,(X(I,J,K),I=1,5),C(J,K)
78 FORMAT(12X,I1,2X,5F5.3,2X,F5.3)
79 CONTINUE
80 WRITE(6,81)(R(I,K),I=1,5),N(K)
81 FORMAT(10X,5F5.3,2X,F5.2,/,/)
82 CONTINUE
```

APPENDIX G (continued)

```
83 WRITE(6,84)(TC(J),J=1,5),(TR(I),I=1,5),(N(K),K=1,7)

84 FORMAT(10X,17F10.4)

85 WRITE(6,86)TSS,SSR,XMSR,FREP,SST,XMST,FMST,SSN,XMSN,FNUC,SSC,XMSC,
   FCON,SSC1,SSC1,FCON1,SSC2,SSC2,FCON2,SSC3,SSC3,FCON3,SSC4,SSC4,FCON4,
   SSNXC,SMSNXC,FNXC,SSE,XMSE

86 FORMAT(10X,30F15.4)

   STOP
```

---

data is read into program according to format outlined in statements 3 and 4.